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Luciemaga Street, Carlsbad, CA 92009 (US). FEITELSON, Jerald, S. [—/US]; 4387 Mistral Place, San Diego, CA 92130 (US). EROSHKIN, Alexy, M. [—/US]; 3803 Ruette San Rafael, San Diego, CA 92130 (US).

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(74) Agents: LLOYD, Jeff et al.; Saliwanchik, Lloyd & Saliwanchik, Suite A-1, 2421 N.W. 41st Street, Gainesville, FL 32606 (US).

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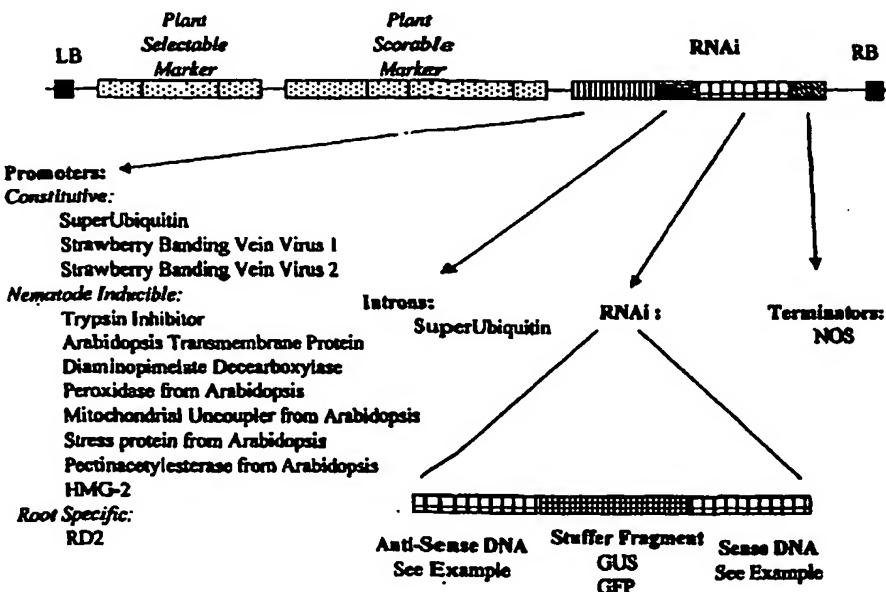
(71) Applicant (for all designated States except US): AKKADIX CORPORATION [US/US]; 4204 Sorrento Valley Blvd., Suite A, San Diego, CA 92121-1412 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): MUSHEGIAN, Aracady, R. [—/US]; 3987 Santa Nella Place, San Diego, CA 92130 (US). TAYLOR, Christopher, G. [—/US]; 2910-A

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(54) Title: MATERIALS AND METHODS FOR THE CONTROL OF NEMATODES



(57) Abstract: The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides RNAi molecules, polynucleotide sequences, and methods of using these sequences in nematode control.

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## DESCRIPTION

### MATERIALS AND METHODS FOR THE CONTROL OF NEMATODES

#### Background of the Invention

[0001] Plant parasitic nematodes, such as root-knot nematodes (*Meloidogyne* species) and cyst nematodes (*Globodera* and *Heterodera*), attack nearly every food crop, and are among the world's most damaging agricultural pests. For example, root-knot nematodes parasitize more than 2,000 plant species from diverse plant families and represent a tremendous threat to crop production world-wide. These biotrophic pathogens have evolved highly specialized and complex feeding relationships with their hosts.

[0002] Nematodes cause millions of dollars of damage each year to turf grasses, ornamental plants, and food crops. Efforts to eliminate or minimize damage caused by nematodes in agricultural settings have typically involved the use of soil fumigation with materials such as chloropicrin, methyl bromide, and dazomet, which volatilize to spread the active ingredient throughout the soil. Such fumigation materials can be highly toxic and may create an environmental hazard. Various non-fumigant chemicals have also been used, but these too create serious environmental problems and can be highly toxic to humans.

[0003] Some research articles have been published concerning the effects of  $\delta$ -endotoxins from *B. thuringiensis* species on the viability of nematodes. See, for example, Bottjer, Bone and Gill ([1985] *Experimental Parasitology* 60:239-244); Ignoffo and Dropkin (Ignoffo, C.M., Dropkin, V.H. [1977] *J. Kans. Entomol. Soc.* 50:394-398); and Ciordia, H. and W.E. Bizzell ([1961] *Jour. of Parasitology* 47:41 [abstract]). Several patents have issued describing the control of nematodes with *B.t.* See, for example, U.S. Patent Nos. 4,948,734; 5,093,120; 5,281,530; 5,426,049; 5,439,881; 5,236,843; 5,322,932; 5,151,363; 5,270,448; 5,350,577; 5,667,993; and 5,670,365. The development of resistance by insects to *B.t.* toxins is one obstacle to the successful use of such toxins.

[0004] The pesticidal activity of avermectins is well known. The avermectins are disaccharide derivatives of pentacyclic, 16-membered lactones. They can be divided into four major compounds: A<sub>1a</sub>, A<sub>2a</sub>, B<sub>1a</sub>, and B<sub>2a</sub>; and four minor compounds: A<sub>1b</sub>, A<sub>2b</sub>, B<sub>1b</sub>, and B<sub>2b</sub>. The isolation and purification of these compounds is also described in U.S. Patent No. 4,310,519, issued January 12, 1982. Avermectin B<sub>2a</sub> is active against the root-knot nematode, *Meloidogyne incognita*. It is reported to be 10-30 times as potent as commercial contact nematicides when incorporated into soil at 0.16-0.25 kg/ha (Boyce Thompson Institute for Plant Research 58th Annual Report [1981]; Putter, I. et al. [1981] "Avermectins: Novel Insecticides, Acaracides, and Nematicides from a Soil Microorganism," *Experientia* 37:963-964). Avermectin B<sub>2a</sub> is not toxic to tomatoes or cucumbers at rates of up to 10 kg/ha.

[0005] Fatty acids are a class of natural compounds which occur abundantly in nature and which have interesting and valuable biological activities. Tarjan and Cheo (Tarjan, A.C., P.C. Cheo [1956] "Nematocidal Value of Some Fatty Acids," Bulletin 332, Contribution 884, Agricultural Experiment Station, University of Rhode Island, Kingston, 41 pp.) report the activity of certain fatty acids against nematodes. In 1977 Sitaramaiah and Singh (Sitaramaiah, K., R.S. Singh [1977] *Indian J. Nematol.* 7:58-65) also examined the response of nematodes to fatty acids. The results of these tests with short chain acids were equivocal, showing nematode-inhibitory action in some instances and stimulatory activity in other instances. Phytotoxicity of these acids was observed at higher concentrations. The short chain fatty acids were also examined by Malik and Jairajpuri (Malik, Z., M.S. Jairajpuri [1977] *Nematol. medit.* 12:73-79), who observed nematode toxicity at high concentrations of the fatty acids.

[0006] Notwithstanding the foregoing (some of the limitations of and problems associated with these approaches are discussed above), there is a need for safe and effective alternatives for controlling nematodes.

[0007] One method for disrupting normal cellular processes is by the use double-stranded interfering RNA (RNAi), or RNA-mediated interference (RNAi). When RNAi corresponding to a sense and antisense sequence of a target mRNA is introduced into a cell, the targeted mRNA is degraded and protein translation of that message is stopped. Although not yet fully understood, the mechanism of this post-transcriptional gene

silencing appears to be at least partially due to the generation of small RNA molecules, about 21 - 25 nucleotides in length, that correspond to the sense and antisense pieces of the RNAi introduced into the cell (Bass, B. L. [2000] "Double-stranded RNA as a template for gene silencing" *Cell* 101:235-238).

[0008] The specificity of this gene silencing mechanism appears to be extremely high, blocking expression only of targeted genes, while leaving other genes unaffected. A recent example of the use of RNAi; to inhibit genetic function in plants used *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* (Chuang, C.-F. and E. M. Meyerowitz [2000] "Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*" *Proc. Natl. Acad. Sci. USA* 97:4985-4990). Chuang *et al.* describe the construction of vectors delivering variable levels of RNAi targeted to each of four genes involved in floral development. Severity of abnormal flower development varied between transgenic lines. For one of the genes, AGAMOUS (AG), a strong correlation existed between declining accumulation of mRNA and increasingly severe phenotypes, suggesting that AG-specific endogenous mRNA is the target of RNAi.

#### Brief Summary of the Invention

[0009] The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides polynucleotide sequences that encode nematode genes, RNAi that selectively targets mRNA transcripts of these essential nematode genes, and methods of using these sequences in nematode control strategies. Such sequences for use according to the subject invention are summarized in Appendix 1. RNAi molecules disclosed herein can be used to inhibit the expression of one or more of these genes in nematodes.

Brief Description of the Drawings

[00010] **Figure 1:** Modular Binary Construct System (MBCS): A series of six, 8-base cutter restriction enzyme sites has been placed between the left and right Ti borders of a previously created kan<sup>R</sup>/tet<sup>R</sup> binary plasmid.

[00011] **Figure 2:** An exemplary shuttle vector created for cloning of useful DNA fragments by containing the multi-cloning site (MCS) of a modified Bluescript plasmid flanked by 8-base restriction sites.

[00012] **Figure 3:** An exemplary shuttle vector with exemplary inserts.

[00013] **Figure 4:** A suggested RNAi binary vector with exemplary inserts.

[00014] **Figure 5:** Exemplary selectable markers for MBCS.

[00015] **Figure 6:** Exemplary scorable markers for MCBS.

[00016] **Figure 7:** Exemplary RNAi binary vector.

[00017] **Figure 8:** Exemplary RNAi shuttle vector.

Brief Description of the Sequences

[00018] Brief Description of the Sequences can be found in Appendix I.

Detailed Disclosure of the Invention

[00019] The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides polynucleotide sequences and methods of using these sequences in nematode control strategies. A preferred method for controlling nematodes according to the subject invention provides materials and methods for controlling nematodes by using double-stranded interfering RNA (RNAi), or RNA-mediated interference (RNAi). The terms RNAi and RNAi are used interchangeably herein unless otherwise noted.

[00020] In one embodiment of the invention, RNAi molecules are provided which are useful in methods of killing nematodes and/or inhibiting their growth, development, parasitism or reproduction. RNAi molecules of the invention are also useful for the regulation of levels of specific mRNA in nematodes.

[00021] dsRNA (RNAi) typically comprises a polynucleotide sequence identical to a target gene (or fragment thereof) linked directly, or indirectly, to a polynucleotide

sequence complementary to the sequence of the target gene (or fragment thereof). The dsRNA may comprise a polynucleotide linker (stuffer) sequence of sufficient length to allow for the two polynucleotide sequences to fold over and hybridize to each other; however, a linker sequence is not necessary. The linker (stuffer) sequence is designed to separate the antisense and sense strands of RNAi significantly enough to limit the effects of steric hindrances and allow for the formation of dsRNA molecules.

[00022] RNA containing a nucleotide sequence identical to a fragment of the target gene is preferred for inhibition; however, RNA sequences with insertions, deletions, and point mutations relative to the target sequence can also be used for inhibition. Sequence identity may be optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, *Sequence Analysis Primer*, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a fragment of the target gene transcript.

[00023] As disclosed herein, 100% sequence identity between the RNA and the target gene is not required to practice the present invention. Thus the invention has the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence.

[00024] RNA may be synthesized either *in vivo* or *in vitro*. Endogenous RNA polymerase of the cell may mediate transcription *in vivo*, or cloned RNA polymerase can be used for transcription *in vivo* or *in vitro*. For transcription from a transgene *in vivo* or an expression construct, a regulatory region (e.g., promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the RNA strand (or strands). Inhibition may be targeted by specific transcription in an organ, tissue, or cell type; stimulation of an environmental condition (e.g., infection, stress, temperature, chemical inducers); and/or engineering transcription at a developmental stage or age. The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus. RNA

may be chemically or enzymatically synthesized by manual or automated reactions. The RNA may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (e.g., T3, T7, SP6). The use and production of an expression construct are known in the art (see, for example, WO 97/32016; U.S. Pat. Nos. 5,593,874; 5,698,425; 5,712,135; 5,789,214; and 5,804,693; and the references cited therein). If synthesized chemically or by *in vitro* enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the RNA may be used with no or a minimum of purification to avoid losses due to sample processing. The RNA may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

[00025] Preferably and most conveniently, RNAi can be targeted to an entire polynucleotide sequence of a gene set forth herein. Preferred RNAi molecules of the instant invention are highly homologous or identical to the polynucleotides summarized in Appendix 1. The homology is preferably greater than 90% and is most preferably greater than 95%.

[00026] Fragments of genes can also be targeted. These fragments are typically in the approximate size range of about 20 nucleotides. Thus, targeted fragments are preferably at least about 15 nucleotides. In certain embodiments, the gene fragment targeted by the RNAi molecule is about 20-25 nucleotides in length. However, other size ranges can also be used. For example, using a *C. elegans* microinjection assay, RNAi "fragments" of about 60 nucleotides with between 95 and 100% identity (to a nematode gene) were determined to cause excellent inhibition.

[00027] Thus, RNAi molecules of the subject invention are not limited to those that are targeted to the full-length polynucleotide or gene. The nematode gene product can be inhibited with a RNAi molecule that is targeted to a portion or fragment of the exemplified polymucleotides; high homology (90-95%) or identity is also preferred, but not necessarily essential, for such applications.

[00028] The polynucleotide sequences identified in Appendix A and shown in the Sequence ID listing are from genes encoding nematode proteins having the functions

shown in Appendix 1. The genes exemplified herein are representative of particular classes of proteins which are preferred targets for disruption according to the subject invention. These classes of proteins include, for example, proteins involved in ribosome assembly; neurol transmitter receptors and ligands; electron transport proteins; metabolic pathway proteins; and protein and polynucleotide production, folding, and processing proteins.

[00029] Genetic regulatory sequences, such as promoters, enhancers, and terminators, can be used in genetic constructs to practice the subject invention. Such constructs themselves can also be used for nematode control. Various constructs can be used to achieve expression in specific plant tissues (by using root specific promoters, for example) and/or to target specific nematode tissues (by using targeting elements or adjacent targeting sequences, for example).

[00030] In a specific embodiment of the subject invention, plant cells, preferably root cells, are genetically modified to produce at least one RNAi that is designed to be taken up by nematodes during feeding to block expression (or the function of) of a target gene. As is known in the art, RNAi can target and reduce (and, in some cases, prevent) the translation of a specific gene product. RNAi can be used to reduce or prevent message translation in any tissue of the nematode because of its ability to cross tissue and cellular boundaries. Thus, RNAi that is contacted with a nematode by soaking, injection, or consumption of a food source will cross tissue and cellular boundaries. RNAi can also be used as an epigenetic factor to prevent the proliferation of subsequent generations of nematodes.

[00031] Nematode polynucleotide sequences disclosed herein demonstrate conserved nucleotide motifs among different nematode genera. Conserved nucleotide motifs strongly suggest that these sequences are associated with viability and/or parasitism and are functionally conserved and expressed in both *Meloidogyne incognita* (root-knot nematode) and *Globodera rostochiensis* and *Globodera pallida* (potato cyst nematodes). The use of these polynucleotides, and RNAi inhibitors thereof, is advantageous because such RNAi can be designed to have broad RNAi specificity and are thus useful for controlling a large number of plant parasitic nematodes *in planta*. Because the genes identified in this disclosure are associated with nematode survival

and/or parasitism, RNAi inhibition of these genes (arising from contacting nematodes with compositions comprising RNAi molecules) prevents and/or reduces parasitic nematode growth, development, and/or parasitism.

[00032] Methods of the subject invention include the transformation of plant cells with genes or polynucleotides of the present invention, which can be used to produce nematode inhibitors or RNAi in the plants. In one embodiment, the transformed plant or plant tissue can express RNAi molecules encoded by the gene or polynucleotide sequence introduced into the plant. Other nematode inhibitors contemplated by the invention include antisense molecules specific to the polynucleotide sequences disclosed herein. The transformation of plants with genetic constructs disclosed herein can be accomplished using techniques well known to those skilled in the art and can involve modification of the gene(s) to optimize expression in the plant to be made resistant to nematode infection and infestation. Furthermore, it is known in the art that many tissues of the transgenic plants (such as the roots) can be targeted for transformation.

[00033] RNA-mediated interference (RNAi) of gene expression. Several aspects of root-knot nematode biology make classical genetic studies difficult with this organism. Since root-knot nematodes reproduce by obligatory mitotic parthenogenesis, the opportunity to perform genetic crosses is not available. Microinjection of RNAi can be used to manipulate gene expression in *C. elegans* (Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. [1998] "Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*" *Nature* 391:806-811). Microinjecting (into adult nematodes) RNAi can turn off specific genes in progeny worms complementary to the coding region of the genes. Moreover, gene inhibition occurs in progeny when RNAi is injected into the body cavity of the adult, indicating the ability of the RNAi to cross cellular boundaries. This RNAi injection method provides a molecular genetic tool that allows for analysis of gene function in root-knot nematodes.

[00034] RNAi can be taken up by *C. elegans* by simply soaking the nematodes in a solution RNAi. This results in targeted inhibition of gene expression in the nematode (Maeda, I., Y. Kohara, M. Yamamoto and A. Sugimoto [1999] "RNAi screening with a non-redundant cDNA set" International Worm Meeting, Madison, WI, abstract 565). Nematodes fed *E. coli* expressing RNAi also demonstrate targeted and

heritable inhibition of gene expression (Sarkissian, M., H. Tabara and C. C. Mello [1999] "A mut-6 screen for RNAi deficient mutants" International Worm Meeting, Madison, WI, abstract 741; Timmons, L. and A. Fire [1998] "Specific interference by ingested dsRNA" *Nature* 395:854; WO 99/32619, hereby incorporated by reference in its entirety).

[00035] Accordingly, one aspect of the instant invention is directed to the control of nematodes comprising contacting nematodes with compositions comprising RNAi molecules specific to the nematode genes disclosed herein. The contacting step may include soaking the nematodes in a solution containing RNAi molecules, feeding nematodes RNAi molecules contained in microbes or plant cells upon which the nematode feeds, or injecting nematodes with RNAi. Nematodes can also be "contacted" and controlled by RNAi expressed in plant tissues that would be consumed, ingested, or frequented by nematodes.

[00036] The RNAi molecules provided to the nematodes may be specific to a single gene. A "cocktail" of RNAi molecules specific to various segments of a single gene can also be used. In addition, a "multigene cocktail" of RNAi molecules specific to two or more genes (or segments thereof) may be applied to the nematodes according to the subject invention.

[00037] In addition to RNAi uptake mediated by transgenic plants, nematodes can be directly transformed with RNAi constructs of cDNAs encoding secretory or other essential proteins to reduce expression of the corresponding gene. The transgenic animals can be assayed for inhibition of gene product using immunoassays or for reduced virulence on a host. Progeny of affected worms can also be assayed by similar methods.

[00038] Procedures that can be used for the preparation and injection of RNAi include those detailed by Fire *et al.*, (1998; <ftp://ciwl.ciwemb.edu>). Root-knot nematodes can be routinely monoxenically cultured on *Arabidopsis thaliana* roots growing on Gamborg's B-5/Gelrite® media. This nematode-host pathosystem is ideally suited for these microinjection experiments since limited root galling results in the parasitic stages (late J2 through adult females) developing outside of the root for easy accessibility for injecting. Another advantage is the parthenogenic reproduction of root-knot nematodes, which makes fertilization by males unnecessary for egg production. The RNAi can be injected into the body cavity of parasitic stages of root-knot nematodes

feeding on *A. thaliana* roots using microinjection. Control nematodes can be injected in parallel with only buffer or an unrelated RNAi. Injected nematodes can be monitored for egg production, and the eggs can be collected for the assays described below. Female root-knot nematodes will typically survive and lay more than 250 eggs following 1  $\mu$ l injection of buffer.

[00039] Alternatively, methods are available for microinjecting materials directly into the plant root cells upon which nematodes feed: giant cells or syncytial cells (Böckenhoff, A. and F.M.W. Grunbler [1994] "Studies on the nutrient uptake by the beet cyst nematode *Heterodera schachtii* by *in situ* microinjection of fluorescent probes into the feeding structures in *Arabidopsis thaliana*" *Parasitology* 109:249-254). This provides an excellent test system to screen RNAi molecules for efficacy by directly inhibiting growth and development of the nematode feeding upon the microinjected plant cell, or by reducing fecundity and the ability of said nematode to generate pathogenic or viable progeny.

[00040] There are a number of strategies that can be followed to assay for RNAi gene interference. Inhibition of gene expression by RNAi inhibits the accumulation of the corresponding secretory protein in the esophageal gland cells of transgenic J2 hatched from the eggs produced by the injected nematodes. In the first assay, polyclonal antibodies to the target gene product can be used in immunolocalization studies (Hussey, R. S. [1989] "Monoclonal antibodies to secretory granules in esophageal glands of *Meloidogyne* species" *J. Nematol.* 21:392-398; Borgonie, G, E. van Driessche, C. D. Link, D. de Waele, and A. Coomans [1994] "Tissue treatment for whole mount internal lectin staining in the nematodes *Caenorhabditis elegans*, *Panagrolaimus superbus* and *Acrobeloides maximus*" *Histochemistry* 101:379-384) to monitor the synthesis of the target protein in the gland cells of progeny of the injected nematodes, or in any other nematode tissue that fails to express the essential targeted gene. Interference of endogenous gene activity by the RNAi eliminates binding of the antibodies to secretory granules in the glands, or any other target tissue, of the transgenic nematodes, and can be monitored by these *in situ* hybridization experiments. Control nematodes injected only with the injection buffer can be processed similar to the RNAi treated nematodes.

[00041] Another assay is designed to determine the effect of the RNAi on reducing the virulence of J2 progeny of the injected females. Egg masses from injected females can be transferred singly to *A. thaliana* plates to assess the ability of the transgenic J2 to infect roots. The J2 hatching from the eggs transferred to the plates can be monitored; after 25 days the number of galls with egg laying females can be recorded. The *A. thaliana* roots can also be stained with acid fuschin to enumerate the number of nematodes in the roots. Egg masses from nematodes injected only with the injection buffer can be handled similarly and used as controls. The treatments can be replicated, and the root infection data can be analyzed statistically. These experiments can be used to assess the importance of the target genes in root-knot nematode's virulence or viability. By staining the J2 progeny of the injected females with the antibodies, it can be determined whether RNAi blocks expression of the targeted gene.

[00042] Additional uses of polynucleotides. The polynucleotide sequences exemplified herein can be used in a variety of ways. These polynucleotides can be used in assays for additional polynucleotides and additional homologous genes, and can be used in tracking the quantitative and temporal expression of parasitism genes in nematodes. These polynucleotides can be cloned into microbes for production and isolation of their gene products. Among the many uses of the isolated gene product is the development of additional inhibitors and modifiers. The protein products of the subject polynucleotides can also be used as diagnostic tools. For example, proteins encoded by the parasitism genes, as identified herein, can be used in large scale screenings for additional peptide inhibitors. The use of peptide phage display screening is one method that can be used in this regard. Thus, the subject invention also provides new biotechnological strategies for managing nematodes under sustainable agricultural conditions.

[00043] Antisense technologies can also be used for phytopathogenic nematode control. Antisense technology can be used to interfere with expression of the disclosed endogenous nematode genes. Antisense technology can also be used to alter the components of plants used as targets by the nematodes. For example, the transformation of a plant with the reverse complement of an endogenous gene encoded by a polynucleotide exemplified herein can result in strand co-suppression and gene silencing

or inhibition of a target involved in the nematode infection process. Thus, the subject invention includes transgenic plants (which are preferably made nematode-resistant in this manner, and other organisms including microbes and phages) comprising RNAi or antisense molecules specific to any of the polynucleotides identified herein.

[00044] Polynucleotide probes. DNA possesses a fundamental property called base complementarity. In nature, DNA ordinarily exists in the form of pairs of anti-parallel strands, the bases on each strand projecting from that strand toward the opposite strand. The base adenine (A) on one strand will always be opposed to the base thymine (T) on the other strand, and the base guanine (G) will be opposed to the base cytosine (C). The bases are held in apposition by their ability to hydrogen bond in this specific way. Though each individual bond is relatively weak, the net effect of many adjacent hydrogen bonded bases, together with base stacking effects, is a stable joining of the two complementary strands. These bonds can be broken by treatments such as high pH or high temperature, and these conditions result in the dissociation, or "denaturation," of the two strands. If the DNA is then placed in conditions which make hydrogen bonding of the bases thermodynamically favorable, the DNA strands will anneal, or "hybridize," and reform the original double-stranded DNA. If carried out under appropriate conditions, this hybridization can be highly specific. That is, only strands with a high degree of base complementarity will be able to form stable double-stranded structures. The relationship of the specificity of hybridization to reaction conditions is well known. Thus, hybridization may be used to test whether two pieces of DNA are complementary in their base sequences. It is this hybridization mechanism which facilitates the use of probes of the subject invention to readily detect and characterize DNA sequences of interest.

[00045] The specifically exemplified polynucleotides of the subject invention can themselves be used as probes. Additional polynucleotide sequences can be added to the ends of (or internally in) the exemplified polynucleotide sequences so that polynucleotides that are longer than the exemplified polynucleotides can also be used as probes. Thus, isolated polynucleotides comprising one or more of the exemplified sequences are within the scope of the subject invention. Polynucleotides that have less nucleotides than the exemplified polynucleotides can also be used and are contemplated within the scope of the present invention. For example, for some purposes, it might be

useful to use a conserved sequence from an exemplified polynucleotide wherein the conserved sequence comprises a portion of an exemplified sequence. Thus, polynucleotides of the subject invention can be used to find additional, homologous (wholly or partially) genes.

[00046] Probes of the subject invention may be composed of DNA, RNA, or PNA (peptide nucleic acid). The probe will normally have at least about 10 bases, more usually at least about 17 bases, and may have about 100 bases or more. Longer probes can readily be utilized, and such probes can be, for example, several kilobases in length. The probe sequence is designed to be at least substantially complementary to a portion of a gene encoding a protein of interest. The probe need not have perfect complementarity to the sequence to which it hybridizes. The probes may be labeled utilizing techniques that are well known to those skilled in this art.

[00047] One approach for the use of the subject invention as probes entails first identifying DNA segments that are homologous with the disclosed nucleotide sequences using, for example, Southern blot analysis of a gene bank. Thus, it is possible, without the aid of biological analysis, to know in advance the probable activity of many new polynucleotides, and of the individual gene products expressed by a given polynucleotide. Such an analysis provides a rapid method for identifying commercially valuable compositions.

[00048] One hybridization procedure useful according to the subject invention typically includes the initial steps of isolating the DNA sample of interest and purifying it chemically. Either lysed nematodes or total fractionated nucleic acid isolated from nematodes can be used. Cells can be treated using known techniques to liberate their DNA (and/or RNA). The DNA sample can be cut into pieces with an appropriate restriction enzyme. The pieces can be separated by size through electrophoresis in a gel, usually agarose or acrylamide. The pieces of interest can be transferred to an immobilizing membrane.

[00049] The particular hybridization technique is not essential to the subject invention. As improvements are made in hybridization techniques, they can be readily applied.

[00050] The probe and sample can then be combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs. Thereafter, the membrane is washed free of extraneous materials, leaving the sample and bound probe molecules typically detected and quantified by autoradiography and/or liquid scintillation counting. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong non-covalent bond between the two molecules, it can be reasonably assumed that the probe and sample are essentially identical or very similar. The probe's detectable label provides a means for determining in a known manner whether hybridization has occurred.

[00051] In the use of the nucleotide segments as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include  $^{32}P$ ,  $^{35}S$ , or the like. Non-radioactive labels include, for example, ligands such as biotin or thyroxine, as well as enzymes such as hydrolases or peroxidases, or the various chemiluminescers such as luciferin, or fluorescent compounds like fluorescein and its derivatives. In addition, the probes can be made inherently fluorescent as described in International Application No. WO 93/16094.

[00052] Various degrees of stringency of hybridization can be employed. The more stringent the conditions, the greater the complementarity that is required for duplex formation. Stringency can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*, Stockton Press, New York, NY., pp. 169-170.

[00053] As used herein "moderate to high stringency" conditions for hybridization refers to conditions that achieve the same, or about the same, degree of specificity of hybridization as the conditions "as described herein." Examples of moderate to high stringency conditions are provided herein. Specifically, hybridization of immobilized DNA on Southern blots with  $^{32}P$ -labeled gene-specific probes was performed using standard methods (Maniatis *et al.*). In general, hybridization and subsequent washes were carried out under moderate to high stringency conditions that

allowed for detection of target sequences with homology to sequences exemplified herein. For double-stranded DNA gene probes, hybridization was carried out overnight at 20-25 °C below the melting temperature (Tm) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula from Beltz *et al.* (1983):

[00054]  $T_m = 81.5^\circ\text{C} + 16.6 \cdot \text{Log}[\text{Na}^+] + 0.41(\%G+C) - 0.61(\%\text{formamide}) - 600/\text{length of duplex in base pairs.}$

Washes are typically carried out as follows:

- (1) Twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash).
- (2) Once at  $T_m - 20^\circ\text{C}$  for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

[00055] For oligonucleotide probes, hybridization was carried out overnight at 10-20°C below the melting temperature (Tm) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. Tm for oligonucleotide probes was determined by the following formula from Suggs *et al.* (1981):

[00056]  $T_m (\text{°C}) = 2(\text{number T/A base pairs}) + 4(\text{number G/C base pairs})$

[00057] Washes were typically carried out as follows:

[00058] (1) Twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash).

[00059] (2) Once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

[00060] In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment of greater than about 70 or so bases in length, the following conditions can be used:

Low:	1 or 2X SSPE, room temperature
Low:	1 or 2X SSPE, 42°C
Moderate:	0.2X or 1X SSPE, 65°C
High:	0.1X SSPE, 65°C.

[00061] Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch

can be tolerated. Therefore, polynucleotide sequences of the subject invention include mutations (both single and multiple), deletions, and insertions in the described sequences, and combinations thereof, wherein said mutations, insertions, and deletions permit formation of stable hybrids with a target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence using standard methods known in the art. Other methods may become known in the future.

[00062] The mutational, insertional, and deletional variants of the polynucleotide sequences of the invention can be used in the same manner as the exemplified polynucleotide sequences so long as the variants have substantial sequence similarity with the original sequence. As used herein, substantial sequence similarity refers to the extent of nucleotide similarity that is sufficient to enable the variant polynucleotide to function in the same capacity as the original sequence. Preferably, this similarity is greater than 50%; more preferably, this similarity is greater than 75%; and most preferably, this similarity is greater than 90%. The degree of similarity needed for the variant to function in its intended capacity will depend upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations that are designed to improve the function of the sequence or otherwise provide a methodological advantage.

[00063] PCR technology. Polymerase Chain Reaction (PCR) is a repetitive, enzymatic, primed synthesis of a nucleic acid sequence. This procedure is well known and commonly used by those skilled in this art (see U.S. Patent Nos. 4,683,195; 4,683,202; and 4,800,159; Saiki *et al.*, 1985). PCR is based on the enzymatic amplification of a DNA fragment of interest that is flanked by two oligonucleotide primers that hybridize to opposite strands of the target sequence. The primers are oriented with the 3' ends pointing towards each other. Repeated cycles of heat denaturation of the template, annealing of the primers to their complementary sequences, and extension of the annealed primers with a DNA polymerase result in the amplification of the segment defined by the 5' ends of the PCR primers. Since the extension product of each primer can serve as a template for the other primer, each cycle essentially doubles the amount of DNA fragment produced in the previous cycle. This results in the exponential accumulation of the specific target fragment, up to several million-fold in a

few hours. By using a thermostable DNA polymerase such as *Taq* polymerase, which is isolated from the thermophilic bacterium *Thermus aquaticus*, the amplification process can be completely automated. Other enzymes that can be used are known to those skilled in the art.

[00064] The polynucleotide sequences of the subject invention (and portions thereof such as conserved regions and portions that serve to distinguish these sequences from previously-known sequences) can be used as, and/or used in the design of, primers for PCR amplification. In performing PCR amplification, a certain degree of mismatch can be tolerated between primer and template. Therefore, mutations, deletions, and insertions (especially additions of nucleotides to the 5' end) of the exemplified polynucleotides can be used in this manner. Mutations, insertions and deletions can be produced in a given primer by methods known to an ordinarily skilled artisan.

[00065] The polynucleotide sequences of the instant invention may be "operably linked" to regulatory sequences such as promoters and enhancers. Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is "operably linked" to DNA encoding a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is "operably linked" to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is "operably linked" to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[00066] Polynucleotides and proteins. Polynucleotides of the subject invention can be defined according to several parameters. One characteristic is the biological activity of the protein products as identified herein. The proteins and genes of the subject invention can be further defined by their amino acid and nucleotide sequences. The sequences of the molecules can be defined in terms of homology to certain exemplified sequences as well as in terms of the ability to hybridize with, or be amplified by, certain

exemplified probes and primers. Additional primers and probes can readily be constructed by those skilled in the art such that alternate polynucleotide sequences encoding the same amino acid sequences can be used to identify and/or characterize additional genes. The proteins of the subject invention can also be identified based on their immunoreactivity with certain antibodies.

[00067] The polynucleotides and proteins of the subject invention include portions, fragments, variants, and mutants of the full-length sequences as well as fusions and chimerics, so long as the encoded protein retains the characteristic biological activity of the proteins identified herein. As used herein, the terms "variants" or "variations" of genes refer to nucleotide sequences that encode the same proteins or which encode equivalent proteins having equivalent biological activity. As used herein, the term "equivalent proteins" refers to proteins having the same or essentially the same biological activity as the exemplified proteins.

[00068] It will be apparent to a person skilled in this art that genes within the scope of the subject invention can be identified and obtained through several means. The specific genes exemplified herein may be obtained from root-knot nematodes. Genes, or portions or variants thereof, may also be artificially synthesized by, for example, a gene synthesizer.

[00069] Variations of genes may be readily constructed using standard techniques such as site-directed mutagenesis and other methods of making point mutations and by DNA shuffling, for example. In addition, gene and protein fragments can be made using commercially available exonucleases, endonucleases, and proteases according to standard procedures. For example, enzymes such as *Bal*31 can be used to systematically cut off nucleotides from the ends of genes. In addition, genes that encode fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these proteins. Of course, molecular techniques for cloning polynucleotides and producing gene constructs of interest are also well known in the art. *In vitro* evaluation techniques, such as MAXYGEN's "Molecular Breeding" can also be applied to practice the subject invention.

[00070] Other molecular techniques can also be applied using the teachings provided herein. For example, antibodies raised against proteins encoded by

polynucleotides disclosed herein can be used to identify and isolate proteins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the proteins that are conserved and most distinct from other proteins. These antibodies can then be used to specifically identify equivalent proteins by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to proteins encoded by polynucleotides disclosed herein, or to equivalent proteins, can readily be prepared using standard procedures known in the art. The genes that encode these proteins can be obtained from various organisms.

[00071] Because of the redundancy of the genetic code, a variety of different DNA sequences can encode the amino acid sequences encoded by the polynucleotide sequences disclosed herein. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding proteins having the same, or essentially the same, amino acid sequence. These variant DNA sequences are within the scope of the subject invention. As used herein, reference to "essentially the same" sequence refers to sequences that have amino acid substitutions, deletions, additions, or insertions that do not materially affect biological activity. Fragments retaining the characteristic biological activity are also included in this definition.

[00072] A further method for identifying genes and polynucleotides (and the proteins encoded thereby) of the subject invention is through the use of oligonucleotide probes. Probes provide a rapid method for identifying genes of the subject invention. The nucleotide segments that are used as probes according to the invention can be synthesized using a DNA synthesizer and standard procedures.

[00073] The subject invention comprises variant or equivalent proteins (and nucleotide sequences coding for equivalent proteins or for inhibitors of the genes encoding such proteins) having the same or similar biological activity of inhibitors or proteins encoded by the exemplified polynucleotides. Equivalent proteins will have amino acid similarity with an exemplified protein (or peptide). The amino acid and/or nucleotide identity will typically be greater than 60%. Preferably, the identity will be greater than 75%. More preferably, the identity will be greater than 80%, and even more preferably greater than 90%. Most preferably, the identity will be greater than 95%. RNAi molecules will also have corresponding identities in these preferred ranges. These

identities are as determined using standard alignment techniques for determining amino acid and/or nucleotide identity. The identity/similarity will be highest in critical regions of the protein or gene including those regions that account for biological activity or that are involved in the determination of three-dimensional configuration that is ultimately responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Below is a list of examples of amino acids belonging to various classes

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

[00074] In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not detract from the ability to manage nematode-caused diseases.

[00075] An "isolated" or "substantially pure" nucleic acid molecule or polynucleotide is a polynucleotide that is substantially separated from other polynucleotide sequences which naturally accompany a nucleic acid molecule. The term embraces a polynucleotide sequence which was removed from its naturally occurring environment by the hand of man. This includes recombinant or cloned DNA isolates,

chemically synthesized analogues and analogues biologically synthesized by heterologous systems. An "isolated" or "purified" protein, likewise, is a protein removed from its naturally occurring environment.

[00076] Recombinant hosts. The genes, antisense, and RNAi polynucleotides within the scope of the present invention can be introduced into a wide variety of microbial or plant hosts. Plant cells can be transformed (made recombinant) in this manner. Microbes, for example, can also be used in the application of RNAi molecules of the subject invention in view of the fact that microbes are a food source for nematodes

[00077] There are many methods for introducing a heterologous gene or polynucleotide into a host cell or cells under conditions that allow for stable maintenance and expression of the gene or polynucleotide. These methods are well known to those skilled in the art. Synthetic genes, such as, for example, those genes modified to enhance expression in a heterologous host (such as by preferred codon usage or by the use of adjoining, downstream, or upstream enhancers) that are functionally equivalent to the genes (and which encode equivalent proteins) can also be used to transform hosts. Methods for the production of synthetic genes are known in the art.

[00078] Where the gene or polynucleotide of interest is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, certain host microbes are preferred. Certain microorganism hosts are known to occupy the phytosphere, phylloplane, phyllosphere, rhizosphere, and/or rhizoplane of one or more crops of interest. These microorganisms can be selected so as to be capable of successfully competing in the particular environment (crop and other habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing a polypeptide of interest, and, desirably, provide for improved protection of the protein/peptide from environmental degradation and inactivation.

[00079] A large number of microorganisms is known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., genera *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilus*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*,

*Arthrobacter, Azotobacter, Leuconostoc, and Alcaligenes; fungi, particularly yeast, e.g., genera Saccharomyces, Cryptococcus, Kluyveromyces, Sporobolomyces, Rhodotorula, and Aureobasidium.* Of particular interest are the pigmented microorganisms.

[00080] Methods of the subject invention also include the transformation of plants or plant tissue with genes which encode the RNAi molecules of the present invention. In one embodiment, the transformed plant or plant tissue expresses antisense RNA and/or RNAi. Transformation of cells can be made by those skilled in the art using standard techniques. Materials necessary for these transformations are disclosed herein or are otherwise readily available to the skilled artisan.

[00081] Additional methods and formulations for control of pests. Control of nematode pests using the RNAi molecules of the instant invention can be accomplished by a variety of additional methods that would be apparent to those skilled in the art having the benefit of the subject disclosure. A "cocktail" of two or more RNAi molecules can be used to disrupt one or more of the genes identified herein. The "cocktail" of RNAi molecules may be specific to segments of a single gene or the entire gene. A "multigene cocktail" of RNAi molecules specific to two or more genes (or segments thereof) is also encompassed by the instant invention. In another embodiment of the instant invention, the disclosed RNAi molecules, cocktails, and/or multigene cocktails thereof, may be used in conjunction with other known nematode control agents and methodologies. Such cocktails can be used to combat the development of resistance by nematodes to a certain inhibitor or inhibitors.

[00082] Compositions of the subject invention which comprise RNAi molecules and carriers can be applied, themselves, directly or indirectly, to locations frequented by, or expected to be frequented by, nematodes. Microbial hosts which were transformed with polynucleotides that encode RNAi molecules, express said RNAi molecules, and which colonize roots (e.g., *Pseudomonas, Bacillus*, and other genera) can be applied to the sites of the pest, where they will proliferate and be ingested. The result is control of the pest. Thus, methods of the subject invention include, for example, the application of recombinant microbes to the pests (or their locations). The recombinant microbes may also be transformed with more than one RNAi molecule thereby delivering a "cocktail" of RNAi molecules to the nematode pests. A carrier may be any substance suitable for

delivering the RNAi molecules to the nematode. Acceptable carriers are well known in the art and also are commercially available. For example, such acceptable carriers are described in E.W. Martin's *Remington's Pharmaceutical Science*, Mack Publishing Company, Easton, PA.

[00083] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

[00084] Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

#### Example 1—Production of Hairy Roots for RNAi Testing

[00085] A hairy root assay system was developed for testing the anti-nematode activity of RNAi molecules.

[00086] *Agrobacterium rhizogenes*: Several *Agrobacterium rhizogenes* strains produce hairy roots on a variety of plant species. *A. rhizogenes* strains, A4, 15834, 8196 and LBA4404 demonstrate hairy root development on tomato and sugar beet, with A4 being the most efficient. The *A. rhizogenes* strain K599 demonstrated very efficient formation on transgenic soybean hairy roots and was also effective on sugar beet and *Arabidopsis*. However, strain K599 failed to produce hairy roots on tomato tissues possibly due to hyper-virulence.

[00087] Hairy root production: Transgenic hairy roots were identified by stable GUS expression in tomato, sugar beet, soybean and *Arabidopsis*. The construct pAKK1401 (pNOS / NPT-II / tNOS // pSU / GUS / tNOS) was used to produce hairy roots when transformed into *A. rhizogenes* strains A4 or K599. Transgenic roots were identified by GUS expression.

#### Example 2—Protocol for Electro-competent *Agrobacterium* and Electroporation

[00088] Electro-competent *Agrobacterium* Protocol:

- [00089] 1. Grow *Agrobacterium* overnight in 5 mls LB + antibiotics at 30°C on shaker (for *Agrobacterium rhizogenes* strain K599 no antibiotics are needed).
- [00090] 2. Use the 5 mls of overnight culture to inoculate 500 mls LB + antibiotics at 30°C on shaker. Grow overnight.
- [00091] 3. Add liquid culture in eight 50 ml polypropylene orange cap tubes.
- [00092] 4. Centrifuge 10 min., 4000 rpm, 4°C.
- [00093] 5. Resuspend cells in each tube with 20 mls 10% glycerol (on ice)
- [00094] 6. Centrifuge 10 min., 4000 rpm, 4°C.
- [00095] 7. Resuspend cells in each tube with 10 mls 10% glycerol (on ice).
- [00096] 8. Centrifuge 10 min., 4000 rpm, 4°C.
- [00097] 9. Resuspend cells in each tube with 2 mls 10% glycerol (on ice).
- [00098] 10. Aliquot 50 µl into cold Eppendorf tube and place onto dry ice.
- [00099] 11. Store electro-competent cells at -80°C. These cells can be used for up to two years.

[000100] Electroporations:

- [000101] 1. Add 1 µl to 5 µl of DNA (resuspended in H<sub>2</sub>O and not TE or other buffer) to 50 µl of *Agrobacterium* electrocompetent cells and mix.
- [000102] 2. Transfer 20 µl of DNA/*Agrobacterium* mix to cuvette.
- [000103] 3. Electroporate:  
25µF, 400 Ω resistance, 2.5 volts (0.2cm cuvette) or 1.8 volts (0.1cm cuvette for BioRad electroporator. 330 µF, 4000 kΩ, low w, fast charge rate for BRL Electroporator.
- [000104] 4. Add 1ml of LB and transfer to Eppendorf tube.
- [000105] 5. Shake at 30°C for 2 hours.
- [000106] 6. Centrifuge down cells (2 min. 14 krpm).
- [000107] 7. Plate all onto LB + antibiotics (most *Agrobacterium* strains are naturally streptomycin resistant).

Example 3 – Protocol for Production of Transgenic Hairy Roots on Soybean

[000108] Seed Sterilization. Rinse the soybean seed with 70% ETOH for 2-5 min. Remove and add 20% Clorox and shake for 20-25 min. Rinse 3X with sterile water. Plate the seed, 5 seed per plate, onto  $\frac{1}{2}$  MSB5 + 2% sucrose + 0.2% gel (referred to as  $\frac{1}{2}$  MSB5). Place seed into chamber at 25C, 16/8 photoperiod for 5-7 day (depending on genotype) germination period. After 1 week seedlings can be placed into cold room for longer storage if necessary (not to exceed 2 weeks).

[000109] Agrobacterium Preparation. For Agrobacterium rhizogenes strain K599, take a small sample from frozen glycerol into 25-50 ml of NZYM media with 50 mg/L kanamycin in a 125-250 ml Erlenmyer flask. Place onto shaker at 28-30 °C for 16 - 20 hours. Pour sample into centrifuge tube and centrifuge the bacterium at 4000 rpm for 10 min. Pour off supernatant and re-suspend the pellet with an equal volume of liquid  $\frac{1}{2}$  MSB5 + 200  $\mu$ M acetosyringone. Use pipette to re-suspend the pellet and homogenize the sample (remove all clumps). To determine O.D., prepare a 1:10 dilution by putting 900  $\mu$ l  $\frac{1}{2}$  MSB5 into cuvette and add 100  $\mu$ l of bacterial sample. Determine the O.D.<sub>660</sub> and calculate the volume needed to adjust (dilute) OD to approximately 0.2 for inoculation. Check final O.D.

[000110] Explant Preparation and inoculation. Place a sterile filter paper onto plates of 1/2 MSB5. Cut soybean cotyledons just above the shoot apex and place onto plate. Lightly scar the cotyledon's abaxial surface (flat side, upper surface that reaches toward sun) with a scalpel blade. Cut each cotyledon transversely into 2-3 pieces (no smaller than 1 cm). Add approximately 10 ml of prepared bacterial solution to each plate and allow cotyledons to incubate for 1 hr. Remove the bacteria using a vacuum aspirator fitted with sterile pipette tip, ensure that there is no standing liquid. Orient all explants with abaxial surface up and wrap plates for a 3 day co-culture, 25°C in light (16/8 photoperiod).

[000111] Hairy root selection and maintenance. After 3 day co-culture, wash explants with liquid  $\frac{1}{2}$  MSB5 + 500 mg/L carbenicillin. Transfer the explants abaxial side up to selection media,  $\frac{1}{2}$  MSB5 supplemented with 500 mg/L carbenicillin and 200 mg/L kanamycin. Roots should develop in approximately 2-3 weeks. The roots will form primarily from the cut vascular bundles with other roots developing from the small cuts on cotyledon surface. Remove roots (>1cm in length) and place onto replica media with

transfers to fresh media every 2 weeks to prevent *Agrobacterium* overgrowth. After 6-8 weeks on selection the roots can be moved to media without kanamycin, however carbenicillin must remain in media for several months for continued suppression of *Agrobacterium*. At this stage roots can be used for testing RNAi for nematode control. Sterilized nematodes can be added and observed for RNAi affects.

Example 4 – Testing of RNAi for Plant Parasitic Nematode Control.

[000112] Various types of nematodes can be used in appropriate bioassays. For example, *Caenorhabditis elegans*, a bacterial feeding nematode, and plant parasitic nematodes can be used for bioassay purposes. Examples of plant parasitic nematodes include a migratory endo-parasite, *Pratylenchus scribneri* (lesion), and two sedentary endo-parasites, *Meloidogyne javanica* (root-knot) and *Heterodera schachtii* (cyst).

[000113] *C. elegans*: RNAi vectors can be tested through expression of the RNAi in *E. coli*. *C. elegans* are fed *E. coli* and assayed for their growth by measuring growth of nematodes, production of eggs and viability of offspring. Another approach is to inject dsRNA directly into living nematodes. Finally, soaking nematodes in a solution of *in vitro*-prepared RNAi can quickly establish efficacy of treatment.

[000114] *P. scribneri*: The *P. scribneri* *in vitro* feeding assay uses a corn root exudate (CRE) as a feeding stimulus and both the red dye Amaranth or potassium arsenate as feeding indicators. Feeding is confirmed after seven days by the presence of red stained intestinal cells in live worms exposed to the Amaranth or death of worms exposed to arsenate. This bioassay is used to test soluble toxins or RNAi. *P. scribneri* has also been cultured on wild type roots of corn, rice and *Arabidopsis*, and on *A. rhizogenes*-induced hairy roots of sugar beet and tomato. *P. scribneri* is very valuable in evaluating transgenic hairy roots because of the non-specific feeding of these worms.

[000115] *M. javanica*: Nematode eggs are sterilized using bleach and are used to inoculate hairy roots expressing RNAi. Nematodes are assessed for their growth by measuring knots, egg masses or production of viable eggs. An alternative approach is to microinject dsRNA directly into root feeding sites or into living female nematodes.

[000116] *H. schachtii*: Cultures of this nematode were maintained on sugar beets. Nematode eggs are sterilized using bleach and used to inoculate hairy roots

expressing RNAi. Nematodes can be assessed for their growth by measuring knots, egg masses or production of viable eggs.

Example 5 – Plant Expression Vectors for RNAi

[000117] Modular Binary Construct System (MBCS): An important aspect of the subject disclosure is the Modular Binary Construct System. The MBCS eases the burden of construct development by creating modular pieces of DNA that can be easily added, removed, or replaced with the use of low frequency cutting restriction enzymes (8-base cutters). These constructs are useful for delivery of a variety of genes to plant cells and is not limited to the delivery of RNAi genes. To develop this system; a series of six, 8-base cutter restriction enzyme sites was placed between the left and right Ti borders of a previously created kan<sup>R</sup>/tet<sup>R</sup> binary plasmid (Figure 1). The production of both kan<sup>R</sup> and tet<sup>R</sup> MBCS aids the testing of constructs using different strains of *Agrobacterium rhizogenes* in different plant species. In addition to the MBCS, a series of shuttle vectors were created that aid in the cloning of useful DNA fragments by containing the multi-cloning site (MCS) of a modified Bluescript plasmid flanked by 8-base restriction sites (Figure 2). With six 8-base cutter sites, each site is, preferably, reserved for a particular function (Figures 3 and 4). Because of the close proximity of the *Pme* I and *Sgf* I sites to the left and right border of the binary vector, these sites are, preferably, reserved for gene tagging and enhancer trap experiments. The *Not* I site is, preferably, reserved for plant selectable markers (Figure 5). The *Pac* I site is reserved, preferably, for Plant Scorable Markers (Figure 6). The *Asc* I site is, preferably, reserved for RNAi experiments (Figures 7 and 8), while the *Sbf* I site is, preferably, reserved for anti-nematode proteins. The restriction sites that are denoted in the Figures are, preferably, reserved for the denoted insertions; however, the MBCS binary and shuttle vectors do not require the restriction sites to contain these suggested inserts.

[000118] Plant Selectable Markers for MBCS: To further develop the MBCS, a series of plant selectable markers were added to the MBCS (Figure 5). Plant selectable markers that were added to the MBCS include: pNOS/NPT-II/tNOS (kan<sup>R</sup>), pNOS/Bar/tNOS (basta<sup>R</sup> for dicots), pUBI/Intron-Bar/tNOS (basta<sup>R</sup> for monocots), and pUBI/Intron-PMI/tNOS (mannitol isomerase<sup>R</sup>).

[000119] Reporter Genes for MBCS: Four exemplary reporter genes are used in the MBCS are provided in Figure 6 and Appendix 2. GUS, a nuclear localized GUS, GEP, and the anthocyanin transcriptional activator *pap1C* genes into the MBCS.

[000120] Promoters for MBCS: We cloned several useful constitutive and nematode-inducible promoters (Figures 6, 7 and Appendix 2). Constitutive promoters include the SuperUbiquitin promoter from pine (pSU) and two promoter regions from the Strawberry Banding Vein virus (pSBV<sub>1</sub> and pSBV<sub>2</sub>). Seven nematode-inducible promoters from *Arabidopsis* were also been cloned.

[000121] The following Scorable marker clones have been constructed and placed in the MBCS, NPT-II binary vector (pNOS/NPT-II/tNOS):

Intron/GUS/tNos	Intron/NL-S-GUS/tNOS	Intron/GFP/tNOS
pSU/Intron/GUS/tNOS	pSU/Intron/NL-S-GUS/tNOS	pSU/Intron/GFP/tNOS
pSBV <sub>1</sub> /Intron/GUS/tNOS	pSBV <sub>1</sub> /Intron/NL-S-GUS/tNOS	pSBV <sub>1</sub> /Intron/GFP/tNOS
pSBV <sub>2</sub> /Intron/GUS/tNOS	pSBV <sub>2</sub> /Intron/NL-S-GUS/tNOS	pSBV <sub>2</sub> /Intron/GFP/tNOS
pKT/Intron/GFP/tNOS		
pKA/Intron/GFP/tNOS		

Example 6 – Control of Plant parasitic nematodes using RNAi in planta

[000122] Production of RNAi Vector: The RNAi shuttle vector to be used is adapted from the Modular Binary Construct System (MBCS - See Example 5). RNAi shuttle vectors preferably comprise a promoter, intron, antisense RNAi, stuffer fragment, sense RNAi, and terminator (See Figures 7 and 8 and Appendix 2 for more details). The plant promoter can be constitutive, tissue-specific or nematode-inducible. The intron is necessary to eliminate expression in *Agrobacterium*.

[000123] The anti-sense and sense RNAi molecules comprise nematode-specific sequences and are disclosed herein. These genes are associated with pathogenesis, growth, or other cellular function in nematodes. An exemplary group of RNAi sequences for use in plant/nematode control may be based upon:

- [000124] 1. Genes specific for nematode esophageal gland cells.
- [000125] 2. Genes specific for plant parasitic nematodes but not other free living nematodes.

- [000126] 3. Genes common to all plant parasitic nematodes.
- [000127] 4. Genes common to all nematodes (nematode-specific).
- [000128] 5. Genes specific for important tissues or cell types.
- [000129] 6. Genes from large gene families.
- [000130] 7. Genes involved in nematode signal transduction or other cellular pathways.

[000131] Appropriate RNAi constructs allow for the formation of dsRNA molecules (the sense and antisense strands join to form the dsRNA). The terminator sequence adds a poly-A tail for transcriptional termination. The RNAi shuttle vector can then be subcloned into the MBCS and transformed into *Agrobacterium rhizogenes*.

[000132] Plant Transformation with RNAi Vectors. An exemplary transformation system for generating hairy roots using *Agrobacterium rhizogenes* is provided below. The RNAi vector once introduced into the MBCS can subsequently (as a binary vector) be transformed in *A. rhizogenes* using, for example, the electroporation protocol of Example 2. Once the *A. rhizogenes* is confirmed to contain the plasmid, it is then used in generating hairy roots (See Example 3). Using this protocol transgenic hairy roots expressing RNAi are isolated, cultured and tested.

[000133] Testing of RNAi Vector for Nematode or Plant Pathogen Resistance. RNAi expressing hairy roots can be inoculated with sterilized nematodes. Infested hairy roots can be observed and the effect on nematodes determined. An alternative approach involves the microinjection of RNAi directly into root feeding sites (giant-cells for root-knot nematode, and syncytia for cyst nematodes) or into living female nematodes.

#### Example 7 – Insertion of Genes Into Plants

[000134] One aspect of the subject invention is the transformation of plants with genes encoding proteins of the present invention. Transformation of plants as described herein can be used to improve the resistance of these plants to attack by the target pest.

[000135] Genes, polynucleotides, and/or RNAi molecules as disclosed or suggested herein can be inserted into plant cells using a variety of techniques which are

well known in the art. For example, a large number of cloning vectors, for example, pBR322, pUC series, M13mp series, pACYC184, pMON, etc., are available for preparation for the insertion of foreign genes into higher plants via injection, biolistics (microparticle bombardment), *Agrobacterium tumefaciens*, or *Agrobacterium rhizogenes*-mediated transformation, or electroporation as well as other possible methods. Once the inserted DNA has been integrated into the genome, the genetically modified-cell(s) can be screened via a vector carried-selectable marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G418, bleomycin, hygromycin, chloramphenicol, or bialophos, *inter alia*. The transformed cell will be regenerated into a morphologically normal plant. The transgene(s) in the transgenic plant is relatively stable and can be inherited by progeny plants.

[000136] If a transformation event involves a germ line cell, then the inserted DNA an corresponding phenotypic trait(s) will be transmitted to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

[000137] It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

We claim:

1. An RNAi molecule, optionally comprising a linker, wherein at least one strand of said RNAi is encoded by a DNA sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 139.
2. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 1.
3. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 2.
4. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 3.
5. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 4.
6. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 5.
7. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 6.
8. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 7.
9. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 8.
10. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 9.

11. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 10.

12. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 11.

13. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 12.

14. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 13.

15. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 14.

16. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 15.

17. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 16.

18. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 17.

19. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 18.

20. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 19.

21. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 20.

22. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
21.
23. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
22.
24. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
23.
25. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
24.
26. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
25.
27. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
26.
28. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
27.
29. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
28.
30. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
29.
31. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
30.
32. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
31.

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33. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 32.
34. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 33.
35. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 34.
36. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 35.
37. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 36.
38. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 37.
39. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 38.
40. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 39.
41. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 40.
42. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 41.
43. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 42.

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44. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 43.

45. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 44.

46. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 45.

47. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 46.

48. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 47.

49. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 48.

50. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 49.

51. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 50.

52. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 51.

53. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 52.

54. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 53.

55. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 54.
56. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 55.
57. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 56.
58. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 57.
59. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 58.
60. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 59.
61. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 60.
62. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 61.
63. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 62.
64. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 63.
65. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 64.

66. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 65.
67. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 66.
68. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 67.
69. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 68.
70. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 69.
71. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 70.
72. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 71.
73. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 72.
74. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 73.
75. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 74.
76. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 75.

77. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 76.

78. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 77.

79. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 78.

80. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 79.

81. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 80.

82. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 81.

83. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 82.

84. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 83.

85. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 84.

86. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 85.

87. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 86.

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88. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 87.
89. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 88.
90. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 89.
91. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 90.
92. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 91.
93. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 92.
94. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 93.
95. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 94.
96. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 95.
97. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 96.
98. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 97.

99. An RNAi molecule according to ~~claim~~ 1, wherein said DNA sequence is SEQ ID NO: 98.

100. An RNAi molecule according to ~~claim~~ 1, wherein said DNA sequence is SEQ ID NO: 99.

101. An RNAi molecule according to ~~claim~~ 1, wherein said DNA sequence is SEQ ID NO: 100.

102. An RNAi molecule according to ~~claim~~ 1, wherein said DNA sequence is SEQ ID NO: 101.

103. An RNAi molecule according to ~~claim~~ 1, wherein said DNA sequence is SEQ ID NO: 102.

104. An RNAi molecule according to ~~claim~~ 1, wherein said DNA sequence is SEQ ID NO: 103.

105. An RNAi molecule according to ~~claim~~ 1, wherein said DNA sequence is SEQ ID NO: 104.

106. An RNAi molecule according to ~~claim~~ 1, wherein said DNA sequence is SEQ ID NO: 105.

107. An RNAi molecule according to ~~claim~~ 1, wherein said DNA sequence is SEQ ID NO: 106.

108. An RNAi molecule according to ~~claim~~ 1, wherein said DNA sequence is SEQ ID NO: 107.

109. An RNAi molecule according to ~~claim~~ 1, wherein said DNA sequence is SEQ ID NO: 108.

110. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 109.

111. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 110.

112. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 111.

113. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 112.

114. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 113.

115. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 114.

116. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 115.

117. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 116.

118. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 117.

119. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 118.

120. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 119.

121. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 120.

122. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 121.

123. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 122.

124. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 123.

125. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 124.

126. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 125.

127. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 126.

128. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 127.

129. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 128.

130. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 129.

131. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 130.

132. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 131.

133. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 132.

134. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 133.

135. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 134.

136. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 135.

137. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 136.

138. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 137.

139. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 138.

140. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 139.

141. A transgenic plant or transgenic plant tissue comprising an RNAi molecule according to any of the preceding claims.

142. A method of disrupting cellular processes in a nematode comprising the steps of:

- (a) providing a composition comprising a compound according to any of the preceding claims; and
- (b) contacting a nematode with said composition.

143. An isolated promoter comprising the following nucleotide sequence:

aacagcccaagataaca~~g~~aaaagtcaaagggtttcgaaa  
gaccacttgtgactaaggatcatttcatccataattatctggtagca  
cagactcatgataactgcgaggaacacaagttttacagtgcattc  
aaagacacttctttacggtttcatgaaggagccgacccagaat  
atgtcagagaagctttactgtgggttaatttcattaatctatcca  
ggtggaaaacctcaaggagatctctttctccaaaagacctctacag  
ggcaatcaaaaaactacagaaccagagttttagtgcacagagtagac  
caatctacctgagaatcacgagtaccttccatagtggtggaaaatgat  
gacatccttattccataccactggatttaggttaggactatccatgg  
aaaaattccatggacaagtcatataagaagaccgcaacagtgcagt  
atcttccagagataactgcactcagacctaaaaggataaaagcagta  
tataatcagtgtactaagatcttcgcagattcaaagaagaagcttaa  
ctatgtgtatgacaagataattctaataagcaattattcagaattaa  
tcaaggagaaagaattaataactctttcagaatatgaagcccgcttt  
acaagtggccagctagctatcactgaaaagacagcaagacaatggtg  
tctcgatgcaccagaaccacatcttgcagcagatgtgaagcagcca  
gagtggtccacaagacgcactcagaaaaggcatcttaccgacaca  
aaaaaaagacaaccacagctcatcatccaaacatgttagactgtcgttat  
gcgtcggtgaagataagactgacc~~cc~~caggccagcactaaagaagaa  
ataatgcaagtggcttagctccacttttagcttataattatgttt  
cattattattctctgctttgctcttatataaaagagcttgat~~ttt~~  
cat~~tt~~gaaggcagaggcgaacacacacacagaacccctgcttaca  
aaccatgtatttagctaaaccttttaggag.

144. An isolated promoter comprising the following nucleotide sequence:

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tggtggggacaatggatccggctgcgttagcaacaaggctg  
aaaaagatcaaacagaaacctgtgatcattagcgttggaccaccacc  
aaaacctcctgagccaccaaagcctccagagcctgaaaaaccaaagc  
ctccaccagcacctgaaccaccaaagcatgtatgcaagccacccat  
tgcaacagttgtatgtgtctgttactacctatgaaagtggaaag  
cggctgcaccattcttgagtcatatacgcttaccatagccttcat  
gttaagtccctgtatccatactaattcatatgttctcatgt  
ttttgttattttttctcaaataatgaaatctctgttggcc  
ctccccctgttataatttagtcgttcttgacacaagaagtctcatg  
agttcatgtaaagaaaataaaagttcaaattaaacaccaaatgtt  
tgatataattccataaacctgtgaagcagaaagttagtcgttgc  
ctgaacagagcttagaagtccctgaaggacatatcttcaagtgcta  
ttgggtcgttagcacttttaggcccattaaacttcattgagccattaa  
attatgcaaaacaagaatgagacatatggaaacattagggttctta  
caggaaaaatagaaaaagcagggacaactaaacaaaaattcagaa  
acaagaggcaagtggacgaccacggcgttaagatcaacatgtgggtat  
gtgcatgagaccaagaccattttctcgatccacgcacacttg  
gtctttctttagttgtgcatttttatttagcagaccctct  
cttttttaataggatagtaaaaaatataatgatttttttttgaaa  
cattttgagttaaaacctaaacttatagtaaagcatttttagttagt  
tttccatatacgacatctatcaacatgacccatcaacaaaaatatt  
gatgaaactactttaaatgttttttttttttttttttttttttttt  
ttaaatttagtagttgtgtaatttttttttttttttttttttttttt  
aaatcaaaacagttatatacgtaacttaggagaatgttttatatcgt  
gttcaacacatgattgttagcatatgttaggtgtcgtagacgta  
cataacaatcatcactcgtaatataatcaaagtggttctgagagaaac  
aaagggttatgatttcccaactgcacttagttgttatttttttt  
cacacgtatgcttctgagttctgccccaaagtggaaattaaagcagag  
ttgggagagatcataatttttttttttttttttttttttttttttt  
cgtaaaatgaaaatttttttttttttttttttttttttttttttt  
ctagttatcttttttttttttttttttttttttttttttttttttt  
tttatacacatcatccaaatcgaattgtctaatcttagagatggaaat  
caggatagagccaataagatataatccaaatggaccaccattttctcc  
atgtgtaattcatacaatctgttttttttttttttttttttttttt  
atgtgagcgttttttttttttttttttttttttttttttttttttt  
aagatggtcttttttttttttttttttttttttttttttttttttt  
gattcaatt  
catgaaacttttttttttttttttttttttttttttttttttttttt  
tgcttt  
ctcagaaaaagcccatttttttttttttttttttttttttttttt  
tactgtgcgttttttttttttttttttttttttttttttttttttt  
actcacagtcacagagatctgttttttttttttttttttttttttt  
ttctttccagt.

145. An isolated promoter comprising the following nucleotide sequence:

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146. An isolated promoter comprising the following nucleotide sequence:

147. An isolated promoter comprising the following nucleotide sequence:

tggcaaactgagatataagagggaaagggtatTTcatgcaa  
atTTTTTTTatTTTTTgaatgaatgcAAATTatTTcaaaaaa  
aaaaaaacTggcTacaatcaagTactTCATTCTgagTTTgaaa  
aatCTAAAGACAACAAAAGACTTTacaatTAATAAAAAAATAAA  
AAATACTTTatCactCTcaacgAAATTGTTgatTTataacgtatCT  
CTTGGTAAAACAGCGTTTatTTgacgAAATTGTTataatGAATAA  
AATGATAATAGAAACTAGTGTGGTACGTTAAACCTCTCATTGGC  
AAAATAACGTTATGTATCATGAGTATTGcatacGACAGCGTGTCTT  
AATAGTGTGCTTCAGGAGAAAATATACCAAGTTATTGCTGAAA  
TTACACGCAAATCTGAGGTTGAAATGGCAAATAAAAAACCAATGT  
CATTTCTTAATGTATAGGTCTTTAAATAAAATTGTACACTTT  
TTCACCTGTAAGCGTTCCAAGTGTAGAATGGATAACTAGAAGGGTC  
AAAGGTATAATTTAATAAGCGAAACTCACTTTGCCAAGTGATT  
CACTTCTTACATTTGCTTGTATAGTTACCCAAAAGTGTATATAT  
TCCCTTATACAATTGTTCTATTCTGGATTATAAGGGAAATAAGAA  
AAAAGAAAAGAGAGAGTATATAATAACTTTATAAAAGTGTATGTTA  
GATTCTAATTGTAACGAAAAGTTCAAAGTGAAGAAAACGAAAA  
AGTTTTCTGTTTGTATCTATAGCCAAGAAAGTTCTCAGA  
TTTACAAGAAGTTAACTGAGAAAAACAAAAAAACTTATGAAGCA  
TGAAGACTAATTAACGAGGTGATTAATTGAGACAAATTAAACAT  
CGAATTAAAGTAACATTGGAGGGTTATATGTTATATGTGACA  
TGATAAGTCCGATTCTGACTAATGTATATCTGGAATCTAACATGGA  
AGAATAGAGAACGAAAGCAGAGCCAAAGGTCAACTGCCAGACACGAAT  
CAACAGATTGTGAATGAGACAAATCAATGGTCATAAACCGGTTGGG  
TTAAACCGGCAAGTCATCCTGGCTCAATTCCATTGTTATTCT  
CATGCAAGACCCCTCTGATACAAACCAAGACTCCCATTACAATATTCT  
TTCGATCACGAGCTACTTCTCAAATGTGTTACCTCTTCGTGAC  
TCTTGTGTGTGGTAAAGCTAGTCGAGATGTGTCGGTATATATA  
GGCATAACATACAAATGCGACAAAATAAGTATATTATATTGTTAA  
TTTCTATATTCCATTCTATATGCTATGGCTGGGATTGGACCAAAA  
CCCTAATTCAAGAATAGAATCCAAGATGGATCAAAGAATATAAT  
CTAATGGCTGACCACATTCCGATTAAATTGCTAGTTAATATT  
CTTCCACTACTTTATGCCGAGAAATTGTAATTAGTAAGACAAA  
GAAATACAGATATAAGATGGCTGTAGAAACCAGTAGAGGAATTCT  
TTTCGTGGATAAGTGGATATTAAATAAGAGAATGGTCTTACTCT  
TACAGTGGAAATGGGAATAGTAGCCATTATAATTCTACAGATT  
TATATATGCTATGGTATAAGCTAAATAATAACGTTAACGATT  
TTCAAAAAAATTACAAGTTCTAGAGACTCTTAACGTCGGCAATT  
TATATTCTACTTTACATGACACTTTCAGGAAAAGAAAACTATACTCA  
CTAGCAGATCATTAAATTCTTTCTTCTTGTAGTAAACTTCAGTGT  
TTGTGGTTTTATTGGTGTAGCTAGAAACTTCAGTGT  
GCCAATGGTAGTGCTTGTGATGATGGTCGG.

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148. An isolated promoter comprising the following nucleotide sequence:

caatcaaggtaacgaaggaggatcagcgaaaggatgggcta  
tatttggagttttcctgcgtgtaaagtaatgcggatcttcca  
tgccggacatataactgaagaataaaactcaactcattgtgttctggtg  
tgtttcttctgatcagattcctcggtgcattctgcactttctgctgt  
gggggcttatttataaaaacaagagtagagcgtgtggtaatcttcat  
atctttctacaattccacttccattctctaatttattctcacgtga  
tatacacacactcaatcaactgtatgtactcgatggatgcagcgtgga  
actgatgcattgccccggatgtcacttctatcgggcttactagaaac  
tgtaagtattacaagaaaactcaaaaggattccatttatgcaaaatc  
taagagaaaagctcaactgtggtctttggttacaatttatggatctc  
aagagacaaatgctatgtaaactaattgattttggtcttgataaaca  
ggtagtggaaagtggacaaagctactcaagaactgaagacatcaaca  
atgcttttgc当地atgaagtctcatgggaccgccttccgc当地cttct  
actcaagc当地acaacaacacagagaccatgtgaaagaacatatggtgc  
gatctaaatttgc当地atgtgc当地cacaagaggactgttcaagccat  
ggtagtggcacgc当地gtgatctgc当地gatttgc当地ttgtatg  
tttattttctaccttctagaaagaggtcaaaaagttaatagcttcac  
cgtgagaatgttgc当地taccagattcatgtgctatgtatgataaaaaag  
acaaaagcaacaacaagagttttcttgcttaggttacaagaacaaga  
gtatcgttataaaagtc当地acaacaagattgaaacatattttgtca>ggg  
agtggtagaaatcttcttactctctgc当地tccactaagacaa  
aaaaaaagacttggactttgtctaaaggttttgtggatattattaacca  
agtccctttgc当地aaaagtaatatttttttgc当地tccctttag  
aatttagttaatcttaggtttatattttgttattactttcttggaaaa  
atgatctgtttattcttattctataacttgc当地tccctttag  
acttctacaaaaggattatcagtgaaagtttagtcttactctcacc  
ttccgaaaataaaaacaaaatctgatacttcttagatcaaaccat  
tgattaaaacatcccttattccctacgattctgatcttgc当地tccctttag  
atcatgttaagatcttatttgc当地aaaactgattttcttcttcttctt  
gtaggaaaaataattacttatttgc当地tccatgttgc当地accgt  
ggtagtttagttacttccatcttcttgc当地aaaactgatcttgc当地tccat  
gaaattataatcaaattaaacatcaatatttgc当地aaaactgatcttgc当地tccat  
ggttttatgttttagaaaattccaaatatttgc当地tccat  
agaagcttattcttcaaattatgttgc当地tccat  
aaaaatataaaagtctaaatatttgc当地tccat  
ccttccaaaggctccaaaggctcaattatgttgc当地tccat  
aaaaggttatttgc当地tccat  
caagcatttagtctttaatcttcttcttgc当地tccat  
aatttttaatttgc当地tccat  
tttagctaatccaactccgttcttatttgc当地tccat  
taaatacgttcttcttcccttatttgc当地tccat  
tcttctcatttgc当地tccat .

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149. An isolated promoter comprising the following nucleotide sequence:

150. An isolated promoter comprising the following nucleotide sequence:

52  
151. A transgenic plant or transgenic plant tissue comprising an isolated promoter according to any of claims 143 through 150.

<sup>54</sup>  
APPENDIX 1

SEQ ID NO:	INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTIDE / GENE
1, 2, 3	2293133	glyceraldehyde-3-phosphate-dehydrogenase
4, 5, 6, 7	7143495	Histone H4
8 & 9	7143515	ATP dependent RNA helicase, mRNA sequence
10, 11, 12, 13	7143527	nematode specific
14 & 15	7143602	protein serine-threonine phosphatase 1, catalytic subunit
16 & 17	7143612	40S ribosomal protein S4
18	7143666	cytochrome p450
19, 20, 21, 22	7143675	Neuroendocrine protein 7B2
23, 24, 25	7143839	nematode specific
26	7143863	40S ribosomal protein S17
27 & 28	7144016	vacuolar ATP synthase subunit G
29	7144025	malate dehydrogenase
30 & 31	7144060	J2 pcDNAII Globodera rostochiensis cDNA similar to Bystin, mRNA sequence
32 & 33	7144225	similar to arginine kinase
34	7144354	pyrroline-5-carboxylate reductase

55

SEQ ID NO:	APPENDIX 1 (cont.) INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E/GENE
35, 36, 37, 38	C10	ribosomal protein L18a
39, 40, 41, 42, 43	C118	ribosomal protein S11
44 & 45	C122	ribosomal protein L16/L10E
46 & 47	C127	FMRFamide-related neuropeptide precursor
48	C129	ADP-ribosylation factor 1
49	C130	ribosomal protein L11
50	C137	nematode specific; conserved in <i>C.elegans</i>
51 & 52	C138	ribosomal protein L7
53	C145	ADP/ATP translocase
54 & 55	C148	troponin
56 & 57	C154	calponin
58	C16	translation elongation factor EF1A
59 & 60	C18	40S ribosomal protein S16
61	C27	ubiquitin
62 & 63	C46	nematode specific
64, 65, 66	C48	ribosomal protein S3AE
67	C59	40S ribosomal protein S5/S7

SEQ ID NO:	<u>APPENDIX 1 (cont.)</u>	FUNCTION OF POLYNUCLEOTIDE / GENE
INTERNAL IDENTIFIER		
68	C8	glyceraldehyde 3-phosphate dehydrogenase
69 & 70	C82	60S ribosomal protein I30/L7E
71	C90	glyceraldehyde 3-phosphate dehydrogenase
72	C135	nematode specific
73 & 74	C206	predicted troponin
75	C227	cytochrome P450
76	C238	vacuolar ATP synthase subunit G
77	C246	40S ribosomal protein S4
78	C308	FMRFamide-like neuropeptide precursor
79	C342	ubiquitin
80 & 81	C344	nematode specific; conserved in <i>C.elegans</i>
82, 83, 84, 85	C370	40S ribosomal protein S5/S7
86	C426	nematode specific
87	C458	histone H4
88 & 89	C481	ribosomal protein L30E
90 & 91	C556	nematode specific; conserved in <i>C.elegans</i>

SEQ ID NO:	INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTIDE / GENE
92	C628	ribosomal protein S17E
93 & 94	C665	malate dehydrogenase
95 & 96	C669	malate dehydrogenase
97	C694	ribosomal protein S3AE
98 & 99	C709	ADP/ATP translocase
100 & 101	C714	ADP-ribosylation factor 1
102	C721	calponin
103 & 104	C726	ribosomal protein L11
105	C736	nematode specific
106 & 107	C773	troponin
108	C834	nematode specific
109	C860	bystin
110 & 111	C863	troponin
112 & 113	C883	translation elongation factor eEF-1A
116	C888	40S ribosomal protein S16
117	C898	glyceraldehyde 3-phosphate dehydrogenase
118 & 119	C935	peptidyl-glycine alpha-amidating monooxygenase
120 & 121	C937	calponin
122 & 123	C942	peptidyl-glycine alpha-amidating monooxygenase

SEQ ID NO:	APPENDIX 1 (cont.) INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
124	C954	arginine kinase
125, 126, 127	C969	calponin
128 & 129	7235653	ribosomal protein L18A
130	8005381	neuroendocrine protein
131	7235496	pyrroline-5-carboxyla te reductase
132 & 133	7275710	protein phosphatase pp1-beta catalytic subunit
134	7923685	nematode specific
135	7641370	40S ribosomal protein S11
136 & 137	7923404	nematode specific
138	7797811	ATP-dependent RNA helicase
139	7143613	predicted phospholipase D

## Appendix 2:

### Exemplary genes used for RNAi vectors.

### **Promoters:**

### **Constitutive:**

**Super Ubiquitin from Pine**  
 cccggggaaaaccctcacaaaatcataaaaaaaaattcttatttaattatc aaactctccact acctt  
 tcccaccaacccgttcaatctgtatgttggaaaaaaact aactacatgtataaaaaaaaacta catta  
 ctcttcttaatcatat caaaattgtataaataatatatccact caaggagtctgaagatccactt ggaca  
 aattgcccatagttg gaaagatgttca ccaagtcacaa gattttatcaatg gaaaaatccatc tacc  
 aacttactttcaagaaaatccaaggat tataagataaaaatctatgtatt attaaatgtcaaaa agaaa  
 accaaaagtgaacaaa tattgtatgtaca agtttgcgagagga taagacattggatcgctttaacca ggagg  
 cggaggaaattcccta gacagttaaaag tggccggatcc cggtaaaaaaaga ttaaaaattttttt ttttt  
 agggagtgcttgaat catgtttttat gatggaaataga ttccagccatc aaaaacattcag gacac  
 ctaaaaattttgaagt ttacaaaaata acttggatctac aaaaatccotat cggatttctct aaata  
 taactagaattttca taactttcaag caactccctcccc taacccgtaaaaacttttctacttc acccgt  
 taattacatcccttaagactagataaa gaaaataaagtaa ataaaaatgttca acaaaaaccaaattt  
 ttcttt  
 aaaaaaaaaaaaaaaaacttt  
 gatccctcgacggag aatctttttatc cccgtggttttgttatttttttttttttttttttttttttttttt  
 ggggatgtggatcc tacagacggctttaatcatacgtct cgagaaggctgac cggatgtgcgac cggat  
 gaccctgtataaccc accgacacagcc agccgacacgtat acacgtgtcaatttcttcatatggaaatatgt  
 cgttgttatcccccgc tggtaacgaaacc accgatgtggtgcac aggtgcgtctgttgcgtgtgcgtacccgg  
 gagaagggtctatc caacgctatataatacttcgccttc accgggttacttctcatatcttttcttttgc  
 ttgtatataatcagtgcgatattctcag agagcttttcat tcaacccggg

**Strawberry Banding Vein Virus 1**  
aagcttttcactgtgggttaattcattaatctatccagggtaaaaacctaaggaga  
tctctttctccaaaagacctacaggcaataaaaactacagaaccagagtt  
gtagtgcacagagtagaccaatctacctgagaatcagtagtaccttcttagagtggg  
aaaatgatgacatcatttccataccactggattgaggtaggactatccaatggaa  
aaattccatgggacaagtcatataagaagaccgcaacagtcgagtatcttccagaga  
taactgcactcagacctaaggataaaagcagtatataatcagtgtactaagatct  
tcgcagattcaaagaagaagtt

Strawberry Banding Vein Virus 2  
Gtttaaacacaacagccaaagataacagaaaaagtcaaagggttgcggaaagaccacttgt  
gactaaggatcatttcatccataattatctggtagcacagactcatgataactgcga  
ggaacacaagttcttacagtcgattcaaagacacttctttacggttcattgaa  
aggagccgaccacagaatatgtcagagaagctttcactgtgggtaatttcattaat  
ctatccaggtgaaaacctcaaggagatctcttctccaaaagacctctacagggc  
aatcaaaaactacagaaccagagttttagtgcacagagttagaccaatctacgttag  
aatcagcgttacccatccatggaaaatgtatgcacatcattccataccactg  
gattgaggtaggactatccaatggaaaaattccatgggacaagtcataagaagac  
cgcaacagtccgagtatcttccagagataactgcactcagacctaaaaggataaaagc  
agtatataatcagttactaagatcttcgcagattcaaaaagagaagcttaactatgc  
tgcacagataattctataaagcaatttccatggaaaatcaaggagaaagaatt  
ataaactcttccagaatataagcccgcttacaaagtggccagcttagtactatcactga  
aaagacagcaagacaatgggtctcgatgcaccagaaccacatcttgcagcagatg  
tgaagcagccagagtggccacaagacgcactcagaaaaaggcatcttctaccgcac  
agaaaaaagacaaccacagctcatcatccaaacatgttagactgtcgttatgcgtcgct  
gaagataagactgaccacccaggccagcactaaagaagaaataatgcacatggccat  
ctccacttttagcttataattatgtttcattattattctctgccttgcctat  
ataaagagctttagttcattgaaggcagaggcgaacacacacacagaacacctcc  
tgcttacaaaccatgtatttagctaaacctttaggaggatate

### **Nematode Inducible:**

### Trypsin Inhibitor from *Arabidopsis* (clone#6598343)

ccccggggagcaaagaagaacaccagagaagaagaaaagcactacagagaaaaatgtg  
agcttaagcgctccaacaacacttctctgggagtctaaaggatgtgcacaaaagc  
cttgggtggtagacttccgatatttccaagcatggttatTTTtttagcacaca  
aactatctgaccctcgactggatTTTcttctgcagttgtccaactacattgaaac  
ggatatgcaggcaacatggatcatgaggggccatctcgtaagattaaacaaagtga  
acaggtcactaaggaaaatacagacggtaactggactcggtccaagggttagaaggag  
gactaaagtgcactcagcaactggcgaattcattgcagtttagacctttattcaag  
aaattgtataccaaaagggtctgtcgtctcttgataatgtgcacatgcaagaagaa  
gtcaggaggatatgcgtacgatacttcatcaagctccaggaaagctaaatctgtcg  
acaatgccattaagttagaggaggatacaccatgaatcaagcaagaccaggtaaga  
acttctctatccataaaccatagatggagcgttagaatcttaatccatTTTcgtt  
tttgaggatcattcatggaggttaatgtctgtgtcagccatgggcttggatggcc  
aaagagtctggctgaatggcagtgaaggataaaagagcgttgcacttaagctt  
gtggaaatttcagatggatggatccaaacaatccgtcgatggcagttgttggaa  
cctaaccatccatgtcagcatatcaggatcatcaatggctcaggcgcaggat  
ctcgttggaaatgcgtcatctacttccatggaaagatggaccaatggaaacccacaac  
agtaatagcagcggagatggatcaacaacgcgtatcgtaaaggccagttatagagaa  
gacactgtacgttcaagttcgagccatcagttgggtgtctcagcttacaaagaa  
gttggaaaacgtttaaactgcaggaCgggtcggttgcgtgaagtacttggatgt  
gaagaagaatgggtatcgttttacagattctgtatctcaagaatgtttggagata  
ttacatggtatggaaaacactcggtgaagttctcggtcgatTTTctgcctt  
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acatagacacacacagttatgtatccaggtaatggatgtttatTTTctctaga  
tattatgtcttataataggcatgaaggagaaaagacaatTTTgtatagtggagt  
tcagcagaaaatgtatatgttttgcgtttatatgaatcagagaataaaagttgg  
tggatatactacgttgcataatgttgcacccatTTTcatataagaaaaag  
agaacacttttagttatccctgtgtgcagaatcgatTTTgtatctccatt  
cctgtggaaaccaaacaatgtcaactaaatttgcgtttaatgggtggTTTaaatgc  
aacggaggacttgcgttttagtgggtttggcattatgtgttgcattatgggtt  
tttcccccttatactgttgcataatccatatcttttttttgcgttgcaccc  
agttcatatccatatcttgcgttgcataatgggttgcgttgcaccc  
ttttcatattgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgtt  
ctcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgtt  
ttgttatgggtcggttgcgttgcgttgcgttgcgttgcgttgcgttgcgtt  
taaatattcacaacaccagtgttaaacacatcaacaacactaaaggttagataaa  
caaagagacccggg

**Arabidopsis Transmembrane Protein from Arabidopsis (clone#6468048)**

ccccggaaattggcactttttctgtgggttccaaaagaaaaacgaatcaatatgtgc  
aacaagaagagctccagaagcagtcatcttctaaaatcttaatctaacaacagctca  
agaagaaaaaaattccatagctagagagaacacaaaagtacaagacgacgtcgtaga  
ggcacaaaagtcaaacctgaatggcttaagccgaactgagtggtttactagaccat  
catcagaaaagtctccaagacggtagtcggatgttagatcgtcaagtaattttgg  
tttgttggtctcacgtttcagctgcccattgattcagttgggtttttcccta  
tctctaaaggcccaatttcatttaggttttagtttattgtcattatccttactata  
aaggcttcgccttcgagaatttaggggttctgtctgtcgtcactcaggtt  
tgtgcctcaacgactgttcacttctgtcatttttttttttttttttttttttttttt  
actgtacatttagattattctgtttctcgagctctgtctatagatttgattcttt  
tttggttgtctttttcggtttccaggatcagatcttagctaaattgagacaagctc  
aaaatgaggtacttgacgcatttttttttttttttttttttttttttttttttttt  
ctctgaatcgtgattcagagacgtatgttcttctgtcatatgcataaagtttaatt  
agagaacaatacgtctctgaatcgtgattgtttttggatgtgcgttattgataagct

ttatgatgttaatagtctaggattgacacgaaggtgtctgcagtttcataaatgc  
ctctttactaaggccttaaatttggatgacaatctaaatcttcataaaaaat  
ttaggtgttataagataagattattttgtatggtagtgtctataatgtgggttggc  
atgttgagggtgtcaatgttgttatttgttttagtaatttgcataactct  
gttcttgtggtaatacagaagcctcagagtgaggccgtcgtgaagccatcac  
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ccagatcggtgtgaagaactatgaccctcaaaaggacaagcgttcagtggatctgt  
caagttaccacatatccccgtcttaaaatgaagatctgcatgtcgagatgcca  
gcatgttgaagagggtgatatatctttcatggaaattgatcatttgtgtctgttt  
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ttgtatatgtttctgtatgttagatgtatgtttcgaaatttggtcattgttta  
ttttaggcttcatttcttgatataatattttgttatttcattttgtatctttc  
gttaggtcgagaagatgggggtggaaaacatggatgttggatctctaaaaaaagctaa  
caagaacaagaaaactcgtaagaagcgttgcggaaataccatgtttctggcctc  
tgagtctgtcatttaagcagattctctgttcttggcctgtttaacaaggcagg  
caagttctggctacagctaatattccattttgttcttacatcggtttgatting  
gataggttttagtagtctatttttgtcaatgttttgatacaatgccaatcc  
tttatctgtgagattatgtttcttgcattttgtatgtttcttgcatttt  
ctttacacaggaaaatcccaactctgtgagcaccaggaaatcttggagtc  
gtgaatgaaacaaaggcaacagtgaagttccagctgaagaaggctgtcatggga  
gttgcagttggtaaccttccggg

**Diaminopimelate Decarboxylase from *Arabidopsis*  
(clone #4159709)**

tgctttgatgatggtccggcccg

### Peroxidase from *Arabidopsis* (clone#4006885)

ccccggggcaatcaaggtaacgaaggaggatcagcggaaaggatgggctatattggagt  
ttttcctgcgtgttaagtaatgcattgtgtctgggtgtgtttctgtatcaggattctcgatgc  
ctgcacatttctgtgtggggcttattataaaaacaagagtagagcgtgtggtaa  
tcttcataatcttctacaattccacttccattctctaattattctctcacgtgatata  
acacacactcaatcaactactgtatgtactcgatggatgcagcgtggaaactgtatgcattgc  
cggggatgtcacttctatcgggcttaatagaaaactgtaaagtattacaagaaaactca  
aaaggattccatttatgcaaaatctaagagaaaactactgtgtcttggttacaa  
tttatggatctctcaagagacaatgtatgtaaacttattttgggtttgtata  
aacagggtgagtggaaagtggacaagactactcaagaactgaagacatcaacaatgttt  
ttgccaatgaagtctcatgggaccgtcttccgcattctactcaagcgacaacaa  
cacagagaccaggtaaaagaaacatatggtgcgtatctaattttgtcaagtgcctcaca  
agaggtactgttcaaggccatggtatggcacgcttgcatttgcgatattttggattt  
tgctttgtatgttattttcaccttctagaaaagaggtcaaaaagttaatagcttc  
ccgtgagaatgttggtttccaggatcatgtgtatgatagaaaaaaagacaaagcaa  
acaagagttttttgttaggttacaagaaacaaggatctgttataaagtcaac  
aaagattgaaacatattttgtcaaggggaggttttagaaattttccactcttt  
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attaaccaagtccctttgcaaaagtatattttttcgattcccttttagaa  
tttagttaatctaggcttatattttgttattactttctgtaaaaatgtatctgttta  
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tgaaaggtagtcttacttcaccttccgaaaaataaaaaacaaaatatcgataacttc  
tagatcaaaccaggatgtttaaaaacatccctattccctacgattctgtatcttgagat  
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aaaaataattactatttagtgcattgtgcaccgtaaagggttttagttact  
ctccatcttcttgaagaaagtccggaaaattatccaaataaaatata  
tattgaacacatatactgtatgttttagtttagaaaattccaaatatttatata  
tccttagggaaaaaaagatcttattcttcaaaattattgttagtgcgtttaaaaatatgg  
ataaaaataataagcttaatattaaaactcagttgcatttttttttacctctcca  
agtctccaaaggatccaaatttttttttagtttaattaaacccaaaaagggttttagttact  
acttagcatgcattgtgggttccaaaacccaaagcattacttctttttaatctt  
ttctccaaataagtttttacaattttttaattttttgcattttccctttagtttatct  
tcatccaaatttagtgcataatccaaactccgttttttatttttttccaaatgttttccat  
aaatacgttcttcttcccttattttcatatcactcaccacaaaagttttccat  
cctcatccccgggg

Mitochondrial Uncoupler from *Arabidopsis*  
(clone#42205-9)

63

tcaaattctggggatattgtatgcattttcgaaacatctcaatgtccccaa  
atacaatcgcttatcatatataatcccgtagtttattttatagatagaataata  
tggcgtatcttataatataacataatagaatcgtagatttttttttttt  
ttatatacgataaaattgcaaaatacttataatgtttttatataatgataccat  
tttatagttactaaaaaaagttaagcgataatataatataatcaacttttataac  
aaaaaaagtataacacatggtaaagaaaaaataaaaaatgaagacatgggtgacacgaa  
aatggcactaaatatacatatataatagatagctacaatatcccatcatacacactt  
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tttattgtcaacatgcaattcatatttccgttgaactatttttttttttttt  
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aaaaaaaaaaagggttctgttctggtaaaatgaaaaaagccaaagcgtttttagatag  
aaaatataactgcctcttatttttttttttttttttttttttttttttttttttttt  
cttgcctcttgcgaaaccctaaaccagaagcaccagatttttttttttttttttt  
gagaacaatagaaaaacccttactgtgtctcttaggttttttttttttttttttt  
tttggattt  
cttccatcgattctggcttctctctctcgctctctcgatgtgctaaatcgccgg  
actgatctcaactgtcacctgttccgggg

### Stress protein from *Arabidopsis* (clone #6598614)

## Pectinacetyl esterase from *Arabidopsis*

(clone#6671954)

ccccgggtggatggggacaatggatccgggtctgcgtaqcaacaqqctgaaaaagatta

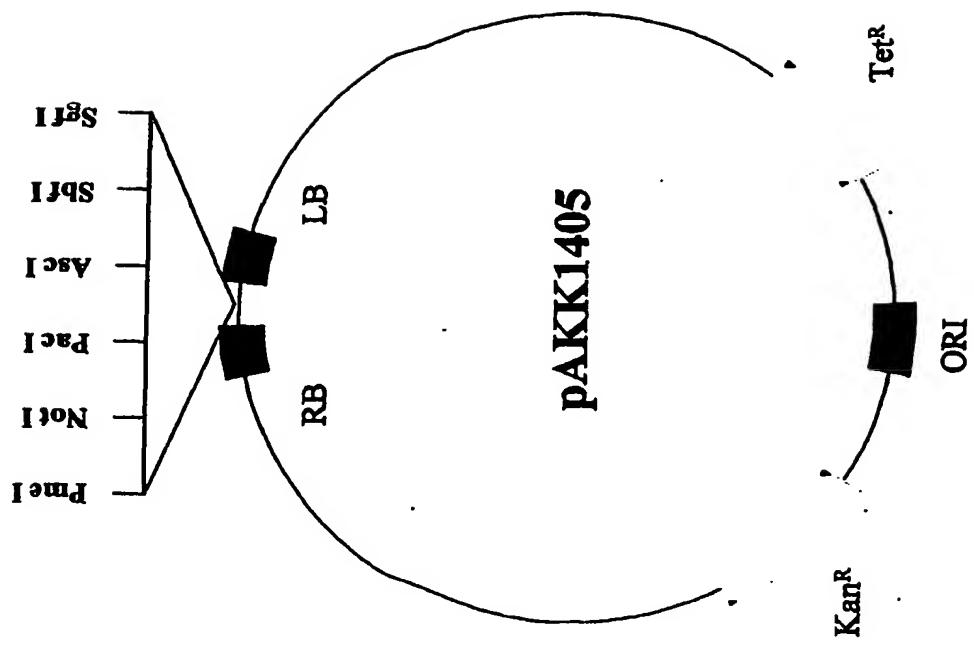


FIG. 1

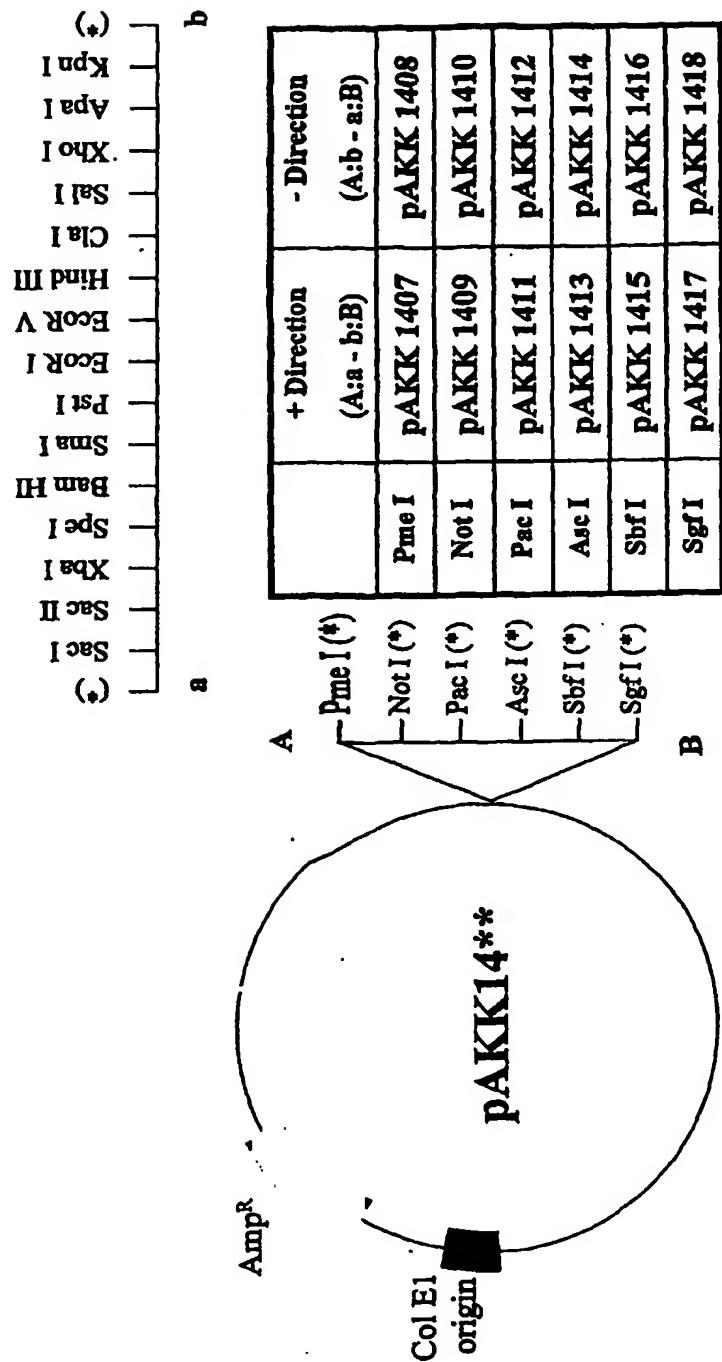


FIG. 2

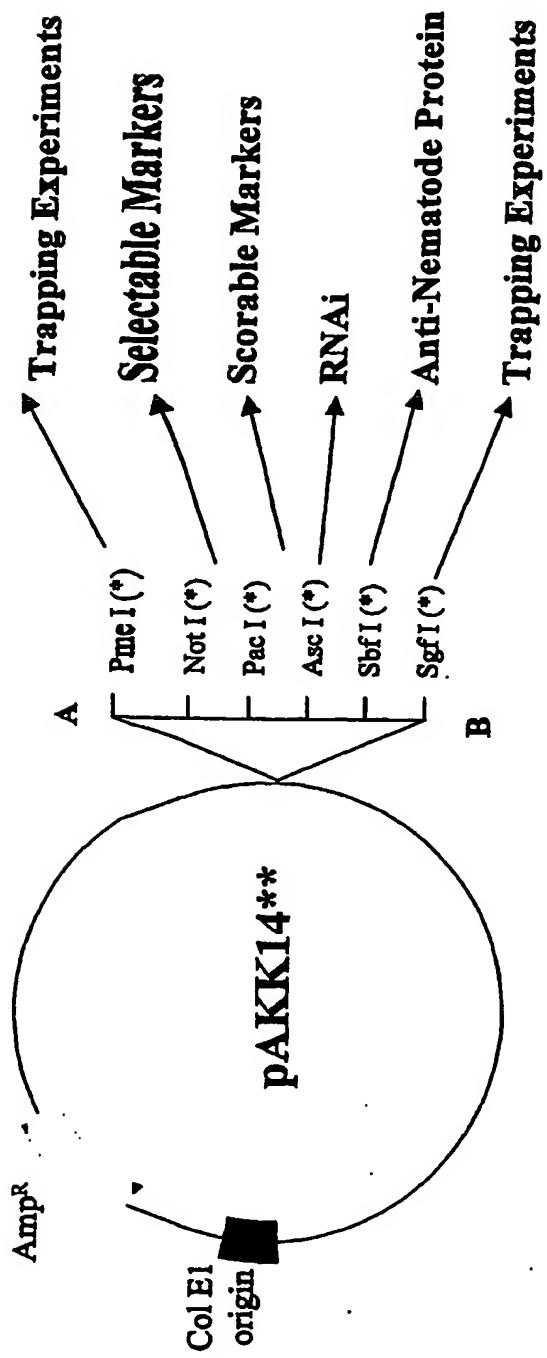


FIG. 3

## Selectable Markers

pNOS / NPT-II / tNOS

pSU / Bar / tNOS

pSU/ Intron / Bar / tNOS

pUBQ3 / Intron / PMI / tNOS

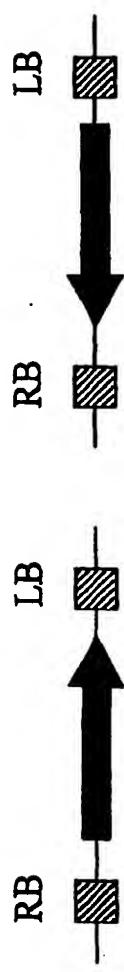
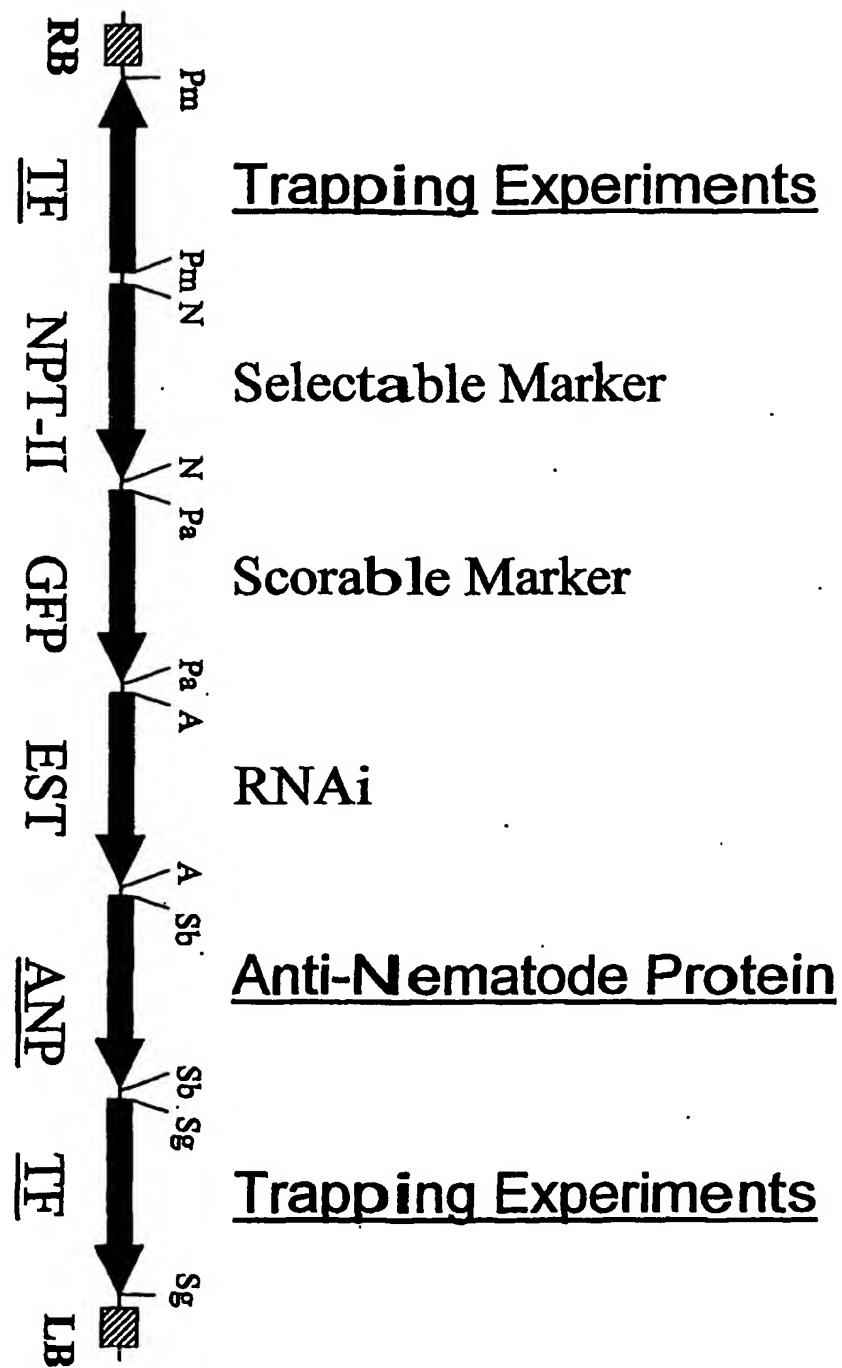
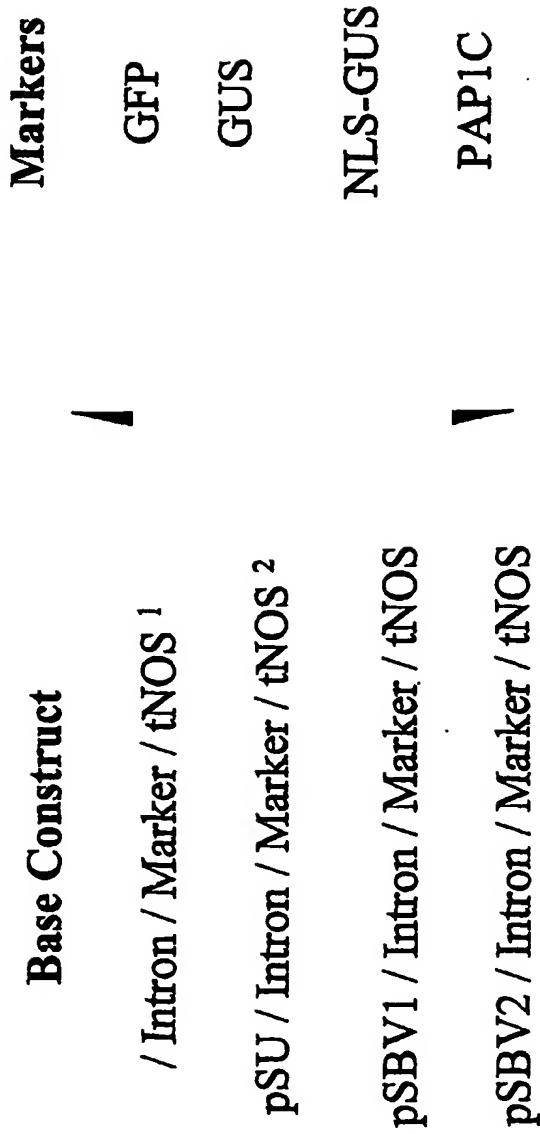


FIG. 5

FIG. 4



## Scorable Markers



- <sup>1</sup> Construct useful for promoter analysis.
- <sup>2</sup> Construct useful for high constitutive expression of genes of interest.

FIG. 6

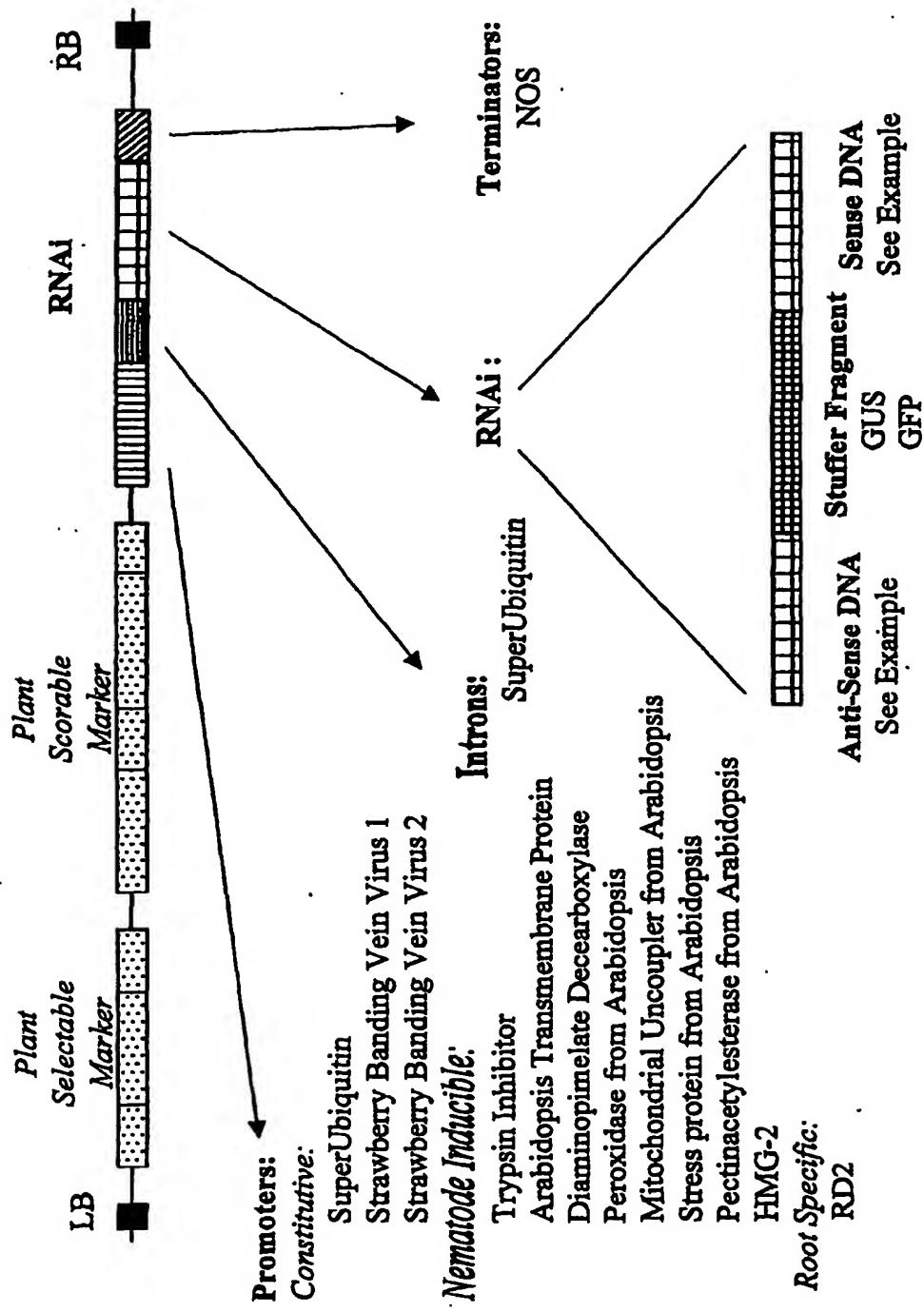


FIG. 7

AKK110P1  
SEQUENCE LISTING

<110> Mushegian, Arcady R.  
Taylor, Christopher G.  
Feitelson, Gerald S.  
Eroshkin, Alexey M.

<120> Materials and Methods for RNAi Control of Nematodes

<130> AKK-110P

<140>  
<141>

<160> 139

<170> PatentIn Ver. 2.1

<210> 1  
<211> 165  
<212> DNA  
<213> Globodera rostochiensis

<400> 1  
gtttgagatt attgactttg catattcca accaagttca tttgaccaat atttcctgc 60  
taaacatagc aaaaatggtg aaaccgaagg tcggcattaa tggcttggc cgcattggc 120  
gcttggcggtt ggcgcgtcgtc gttgagaaggc acaccgttca ggtgg 165

<210> 2  
<211> 342  
<212> DNA  
<213> Globodera rostochiensis

<400> 2  
cgactacatg gtatacatgt tcaactacg a ctcgacccat ggccgcttca atggcaaaat 60  
ttcgacaaggc gcccgaatt tggcgttgc gaaagagggg aaggccacgc acaccatcaa 120  
ggtgttcaac ctcaggacc cggccgagat caaatggct gaggtggcg cggaaatatgt 180  
gatcgagtcc accgggggtgt tcactaccat tgagaaggct tcggcacact tgaaggggg 240  
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caacgaggac aaatatgacc cggccaaggaa caacgtgatt ag 342

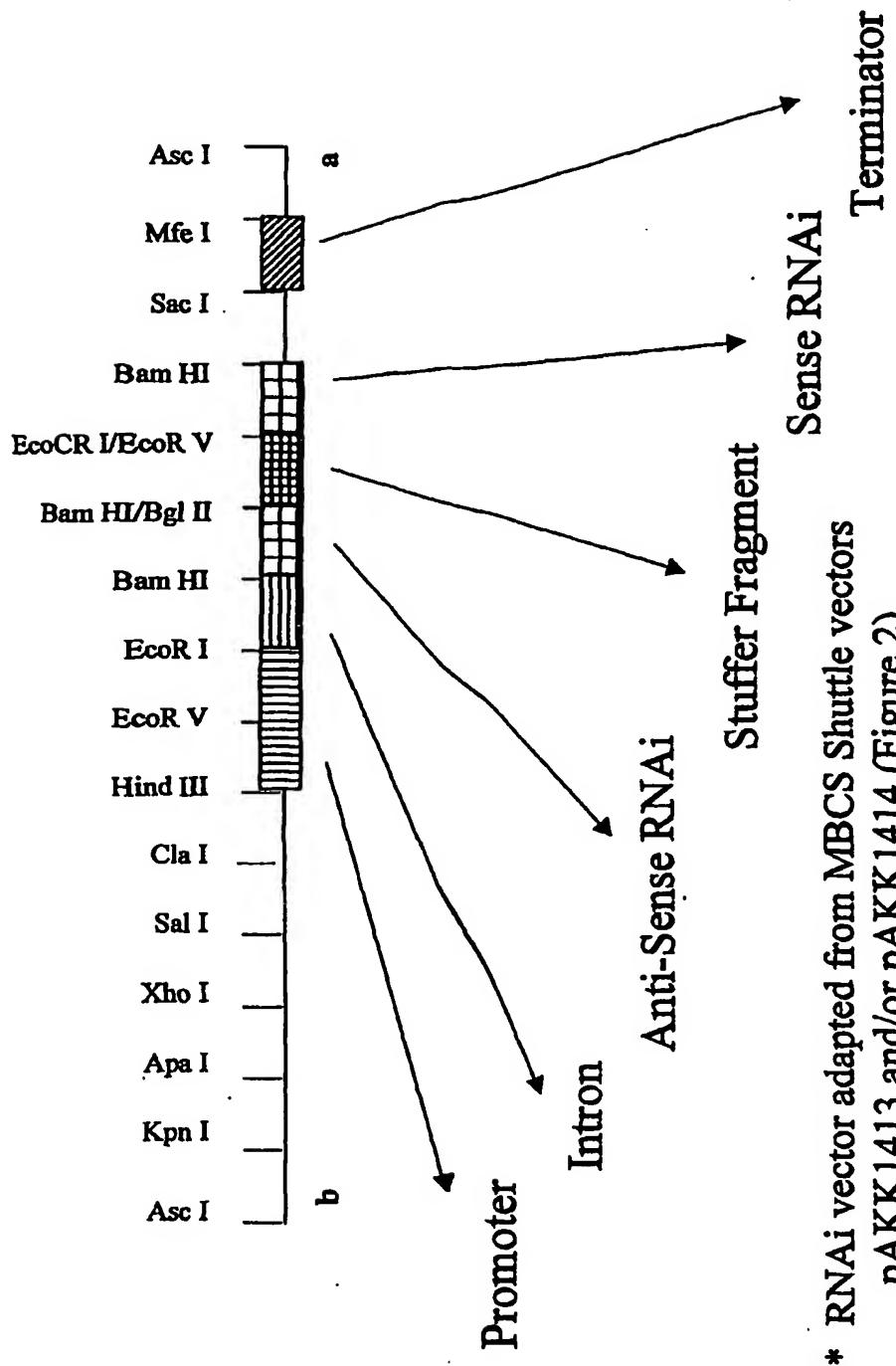
<210> 3  
<211> 205  
<212> DNA  
<213> Globodera rostochiensis

<400> 3  
gaagccggcc tcattggacg ccatcaaggc ggcgggtgaag aaggctgccg aaggaaattt 60  
gaaggccatt tgggttaca cagaggacc a ggtgggttcc acggacttcc ttggagacag 120  
tcgctcgatcg atcttcgacg ctggggcgatg catctcgatgg aacccgact ttgtcaagt 180  
ggtcagctgg tacgacaatg aattt 205

<210> 4  
<211> 167  
<212> DNA  
<213> Globodera rostochiensis

<400> 4  
ttaaacgatt tattcacacg cacggagaaa tgaggattac ctaatttgat tgagtcttc 60  
tcgtccattt gtcattgtg gcccataaga gggccgtttg ggttagttt ttgggtgtcc 120  
ttctccttgc tggctcaacc accgaaggc tacagcgtcc ggccttg 167

<210> 5



\* RNAi vector adapted from MBGS Shuttle vectors  
pAKK1413 and/or pAKK1414 (Figure 2).

FIG. 8

## AKK110P1

<211> 41  
<212> DNA  
<213> Globodera rostochiensis

<400> 5  
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<212> DNA  
<213> Globodera rostochiensis

<400> 6  
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cttaacgcct ccacgacgg 79

<210> 7  
<211> 168  
<212> DNA  
<213> Globodera rostochiensis

<400> 7  
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cagagataggagaaaata gggaaattttg cctcgtgcg aacgtgcc 168

<210> 8  
<211> 330  
<212> DNA  
<213> Globodera rostochiensis

<400> 8  
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gccgataaaag aaagacgaag aggtattggc taagaacacg ccgcacatttgc tgcgtggAAC 180  
gcccggacgt cttttggct taggacgcac tggacatctg aagctgaaag ggcgtcaaatc 240  
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ggaaatcttc aaaatgacgc ctcaggagaa 330

<210> 9  
<211> 136  
<212> DNA  
<213> Globodera rostochiensis

<400> 9  
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tacgtcgacg acgaggctaa gcttacgtt caccgtctcc aacaatacta cgttagactg 120  
aaggaaaatg agaaga 136

<210> 10  
<211> 141  
<212> DNA  
<213> Globodera rostochiensis

<400> 10  
tataaaaata aaatacaaac aataatataa tggctgtttt ttctgtcatg tttcaagttt 60  
ttgttgttca tcactttctt cagcagcgac aatacggcca atccggtgaa agggccaaag 120  
tcaatagctc gtcgggtacc t 141

<210> 11  
<211> 141  
<212> DNA

## AKK110P1

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 17

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tggacggcaa	agtgcgcacc	gagatgcgc	<b>T</b>	tcccggtcg	aataatggat	gtgatctcg	120
ttgagaagac	aaacgaaacg	tttcgtctgg	<b>T</b>	tgtacgtat	gaaggccgt	tttgcattcc	180
atcgaattca	aaagctggag	ggccgatc	<b>A</b>	agtgtgcaa	agtgaagaag	caggccgtcg	240
gggacaagca	ggtccctac	attgtcaca	<b>C</b>	atgacgcgcg	caccattcgc	tacccgaccg	300
ctcatc							306

&lt;210&gt; 18

&lt;211&gt; 528

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 18

gaattcgcac	aacgaattga	agacttgc	<b>C</b>	ggcagaaaaaa	ggactttgc	caaagtgtga	60
ggagcaagca	gacgacctt	cgattgg	<b>T</b>	tttgcgtcc	atgggttgg	agcatcgccc	120
gttcctaccg	tatacaaacg	ctgtataaa	<b>A</b>	rgaaaacaatt	cgattagtca	atttgatccc	180
gttcaatctt	agccatttgg	cgcttgaa	<b>A</b>	tatgcaattt	ggcaattttt	tttgtaagcgc	240
tgggacacca	attgttacgc	aggtcagca	<b>G</b>	tttctgttc	gacgaaaaac	tgtatccgga	300
gcccacatgg	tttttgcggc	aacgtttt	<b>T</b>	ggacgttag	ggccgtttga	agaaaagcga	360
cgaacttatt	gcattttgggg	ttggggaaa	<b>G</b>	gcaatgtgcc	ggcgaagctt	tggccgaat	420
gacacttttt	ctgtttgcgc	ctaatttct	<b>T</b>	tctcgctac	aaagttctcc	cgtccgatcc	480
actgaatctt	ccaagcctga	aaaagtgg	<b>C</b>	ggattatctg	tttacaca		528

&lt;210&gt; 19

&lt;211&gt; 335

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 19

gaattctttg	agaaaagcgg	aattcg	<b>T</b>	tggctataaa	atgattctgt	ggccacgat	60
tttgttgcgt	gctttggaca	ttgcgttc	<b>G</b>	tggcacaat	caaatggat	ttgatcagtc	120
ggcgcgcgt	ttcccccact	cccagt	<b>T</b>	cgatttgatt	tgcgcgaca	tcaatccctt	180
ctccgcgc	ttgggcgttg	gccataaa	<b>T</b>	tatgagcggc	ggtgcgggt	aggcgtcca	240
acagctaggc	cccgaggggc	ccttgagac	<b>G</b>	gcccacacag	gtgaagagtg	acaatgttct	300
ccccgcgtat	tgcgagcctc	caaattcc	<b>T</b>	tccga			335

&lt;210&gt; 20

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 20

ggacggctgc acggaacagt tcgagaaca

**C** tgccgagttt tcgcgcagct ac

52

&lt;210&gt; 21

&lt;211&gt; 190

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 21

gcttggtgt	gcaggaggcac	atgtttact	<b>T</b>	gtccgtcgaa	gaacaaccgc	gaggaggatcg	60
agcaggat	cttggcaattt	ctggccaaac	<b>A</b>	acggactgca	caaatcaatg	attgccaaga	120
aattccat	cttgcgggcg	gaggagcc	<b>C</b>	gcccgtcgaaa	acgctttgt	cgcccggtt	180
cggccaaac	cg						190

&lt;210&gt; 22

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

## AKK110P1

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 <211> 54  
 <212> DNA  
 <213> Globodera rostochiensis  
  
 <400> 23  
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 <210> 24  
 <211> 77  
 <212> DNA  
 <213> Globodera rostochiensis  
  
 <400> 24  
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 aacagacccg aacagaca 77  
  
 <210> 25  
 <211> 439  
 <212> DNA  
 <213> Globodera rostochiensis  
  
 <400> 25  
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 tccatccgt ctcttctaca tcagcaacac aatcacattc cacgccccagt tttatgacac 120  
 acaacgtca gcagcaacat gttttggc aacaacagca gcaacaacag aatttccaac 180  
 aaccggccccc cctatcgatc actcacagcc accaacaaca aaaaacaacca ccacaaggcg 240  
 cacagtcatgatc gttgtcaatg aaaagtggca atgttgcgt tggtgtccg caacaatcg 300  
 agcagcacca ctaccaacag cgacactga cgccactgaa gcacacatcc gcattccca 360  
 cgtccgatcg cttcgatc accaaaacca acagggtgct tccactcccg tcgcagcaag 420  
 gcccacccg cactgatc 439  
  
 <210> 26  
 <211> 539  
 <212> DNA  
 <213> Globodera rostochiensis  
  
 <400> 26  
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 aacggaaaact gtgaagaagg cgtcgccgt cattatttgcg aagtattaccc ccaatttggg 120  
 cctcgacttt cacaccaaca agcgcatttg cgaggagggtg gccattatcc caagcaacg 180  
 gatcgaaac cgaattgcgg gatttacac acatctgtatc aagcgcatttgcgt agctggccc 240  
 tgcgtggc atttccatca aattgcggaa ggaggaggcg gaggcgatc acaatttacat 300  
 gcccggaaatc tcttacctgg atgcgcggaa tcacccatgt atcgcacccg accaagagac 360  
 gaaggatatg gggaaatttgcgtggc tggggcttggc cctcaacttgcgtt gaaatggaaag ggccttgc 420  
 gatcgccggc gctggcgccag gacgtcgatgcgtt agtcaggacca attggcatttttttttggat 480  
 atcatcgatc ttttgcgttgcg atttggatgcgtt taatgcgttgcgtt ataaatttttt 539  
  
 <210> 27  
 <211> 179  
 <212> DNA  
 <213> Globodera rostochiensis  
  
 <400> 27  
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 cggccggaaaa gctgtcgccgca gaaaagatca atgatgcccgg aagcggaaaa gcacagcgac 120  
 ttaagcaggc caaacaagaa gcccaggccg agatcgagca gtatcgncag gagagggag 179



## AKK110P1

&lt;211&gt; 425

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 33

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tacgtccctg acgctgaggc ttacaccctg ttcaagccgt tggtcgaccc gatcatcaac 180
gactaccatg gtggctttgg tccggcagc aagcagccgg caactgaccc ttgtgacggc 240
aaaacgcana tgctgaccgg atctcgaccc cgagggggaa atttatcaat ttgcacacgc 300
gttcgttgcg gccgtttcct ttaagggata cccggttcaa cccgtgcttgc acnaaaggan 360
aactacnittt ggagatggga aacnaaggtc naggggccgtt ttctaacatt ttñaagggn 420
atccct                                              425

```

&lt;210&gt; 34

&lt;211&gt; 581

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 34

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gaattcgttt gagcgaagag ttttgtggtt gacaccgggtt tatggacttt tagcccgta 60
tccttgacgg tccaaagccg cgttcagttc cggtccgtgt tttttaaaag aggccggagag 120
tttgacggtc attccaaagca gccaataaaac caccaaaaacc aaataccccc ccccaatcga 180
tccccccctt ccaattccctc cgcattattc gcattatcaa ttctaattcag cacaaccact 240
gcattcattcc ttccccgacc atacgatgtt aagtggaaact ttgaaaatttgc ttccatcg 300
agccggaaag atggcccaag cattggcaag aggacttatac aattcggggc gataccggc 360
agagaatttg atggcgagtt gtccaaagaa ggacgaggct ttactggagc aatgaaaaaa 420
attgggaatc ggaacgacgc acgacaacac ttgggtcgcg cgagagaacg acgtcatcg 480
attggcggtc aagccgatgc acatcagcaa agtgacgtcg gaaatcgac ccaatttccg 540
gagggaaacat ttgttattt cattgattag gaattacact t                                              581

```

&lt;210&gt; 35

&lt;211&gt; 102

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 35

```

gaattcgttt gagaatttttata ttgacgttta atcagcagcc ataaagcaatg 60
cccatcaaag catccggaga aacattaagg aagtttatttgc                                              102

```

&lt;210&gt; 36

&lt;211&gt; 34

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 36

tgcaaatgt gcaaaccaca cgttcacaa gatg

34

&lt;210&gt; 37

&lt;211&gt; 100

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 37

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tcatgttgcg gccaaatctc gtttctggta ctttacgagc atgctgcgtc gagttaaagaa 60
aacacacggaa gagatcggtt cgtgtcaaga ggttttcgag                                              100

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&lt;210&gt; 38

&lt;211&gt; 176

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 38

## AKK110P1

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 gcgagtatcg ctgatgttac cgagggcg~~gt~~ gccgtgaccc aatgctatcg cgacatggc 120  
 gctcgta~~c~~ ggc~~t~~ctcaggc ggatcga~~tt~~ caaatcatca aagtgc~~aa~~ac ctcaag 176

<210> 39  
 <211> 155

<212> DNA

<213> *Globodera rostochiensis*

<400> 39

gaattccaag tttgaggtat tg~~ttt~~ttat acgatttctt acaa~~at~~gaca gaacaaactg 60  
 agcgcgcgtt ccaaaaacaa ccgatcgt~~tt~~ ttctgaacga caagttcaga acgcaaggga 120  
 ttgggaagaa ggc~~t~~atccaa~~c~~ aaggaccg~~tt~~ actgg 155

<210> 40  
 <211> 35

<212> DNA

<213> *Globodera rostochiensis*

<400> 40

tcctcg~~c~~gag gctattgagg gcata~~at~~at~~at~~ cgaca

35

<210> 41  
 <211> 70

<212> DNA

<213> *Globodera rostochiensis*

<400> 41

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 g~~c~~ggac~~g~~att 70

<210> 42  
 <211> 85

<212> DNA

<213> *Globodera rostochiensis*

<400> 42

tcgtaccaaa atatcg~~c~~gc tatgaga~~aa~~c gccacaaaaa catgtccgtc cactgttc~~gc~~ 60  
 c~~g~~tgc~~t~~ccg agatgtctct ctcgg 85

<210> 43  
 <211> 193

<212> DNA

<213> *Globodera rostochiensis*

<400> 43

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 attctgagtc g~~cc~~caag~~cc~~ca accgcga~~ac~~g gtc~~at~~ttt~~gt~~t at~~gg~~ttc~~ct~~ta att~~tt~~tg~~ct~~g 120  
 ttttcaatt att~~tg~~gtt~~a~~ aatgactg~~aa~~ tttatgatca acgg~~t~~ata~~t~~act agtattctc 180  
 t~~g~~aaaaag~~ct~~ c~~g~~a 193

<210> 44  
 <211> 219

<212> DNA

<213> *Globodera rostochiensis*

<400> 44

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 gaagacgtcc ggcgcgtt~~gt~~t at~~c~~gctat~~a~~ ttaagaacaa g~~cc~~gtatccg aagtcgc~~gt~~t 120  
 tt~~t~~gtgc~~gg~~ t~~g~~taccc~~gc~~ac ccaaaaat~~c~~ g~~c~~at~~ttt~~ga tt~~t~~gggt~~ag~~a aagcgc~~gg~~ca 180  
 ccgtt~~g~~ac~~g~~a attccat~~gc~~ t~~g~~cgt~~gc~~at~~a~~ tgat~~at~~cg~~a~~ 219

AKK110P1

&lt;210&gt; 45

&lt;211&gt; 489

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 45

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aaggacgggt	ttcatatgcg	cgtcagaatc	catccatacc	atgttaattcg	catcaacaaa	120
atgttgcct	gcgctgggtgc	ggaccgtctg	cagactggga	tgcgtgggtc	gttcggaaag	180
cctcaggggac	tctgtggcgc	tgtcagcatc	gtgtatatgc	tgtatgtcagt	gcgtattcgt	240
gaccaacacc	aaagtcacgc	attggaggcg	ttccgtcggt	ctaaattcaa	gttccctgggt	300
cgtcaataca	tctgtttgtc	ccgcaagttgg	ggcttccacca	aattcgatcg	cgaggatatac	360
gagaaatacc	gcaaggagggg	ccgtgttac	cctgacgggt	tgcattgcaa	gttactcaag	420
caacacggac	ccgctgaagg	agtggctcaa	gaaccccatt	taatcttctg	tttgcgttgt	480
gactcttgg						489

&lt;210&gt; 46

&lt;211&gt; 101

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 46

gaattccccc	gctcgagccg	ggttgacgat	gtccctctcc	acccctctc	actgcgttcc	60
gtcctccttc	agccggaaat	tgttcctgtg	gctgttgccg	g		101

&lt;210&gt; 47

&lt;211&gt; 485

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 47

tccaccaaaag	tccatttcgct	gtcgccagtc	catttattcc	acaaaaagat	gattccgtcg	60
tcgttccgat	gacgtcggtt	ggccaaacgt	tgcctccgtc	accgcttca	ctgggtccaa	120
acccgcccgt	ttattttgc	ttcccagaaa	acttgcgtt	ggagcggccc	ttcgtacgagc	180
aaaacgacgg	ctccgaggag	gaattagccg	aagaagcgt	ggaaacgaag	gcgaagaggg	240
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cacaacatt	tgtacgcctc	ggaagggaca	cgcaaaaggca	attcgatgg	aaaatgcaaa	360
gtgaacacgca	acagaaaaag	gcttaaaagca	aacggcggcg	acttttctt	taatgaatgc	420
gcgcccaccc	catgacaatt	cttttgcgt	atgtgttgcg	attttatga	tcggtaaatg	480
taaca						485

&lt;210&gt; 48

&lt;211&gt; 651

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 48

atctgttcaa	gggactgttc	ggcaagaagg	aaatgcgtat	tctgtatggtt	gggttggacg	60
ctgctggaaa	gacgaccatt	ctgtacaatg	taaagctcg	cgaaattgtc	accaccatcc	120
caacaattgg	cttcaacgt	gaaaccgtcg	aatacagaaa	catctcggtc	actgtttggg	180
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gactgtatctt	cgtcgtggac	agcaacgtc	gcgacgtgt	gggcgaggcg	cgtgaagagt	300
tgatgcgtat	gctggcggag	gacgagtgc	gcgacgcgt	tgtgtgggt	ttcgtctaaca	360
aacaggattt	gccaatgcg	atgaacgccg	ccgaaactgac	agacagactt	ggactgcaca	420
acttgcgaaa	ccgcaatgg	tacatccagg	ccacctgcgc	gacttcgggc	gacggactct	480
acgagggtact	ggactggctg	agcaaccac	tcaagaacag	aggctaagct	gggttgggtgt	540
ctgttgcact	tgcccgccga	attgatgacg	attgaattt	tttgcgttgt	tcgcgcgcga	600
gctttttgt	gggacgtccg	attaattttg	ataaattttt	tattccgtgt	t	651

&lt;210&gt; 49

&lt;211&gt; 660

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

AKK110P1

&lt;400&gt; 49

gaattcccaa	gtttgagatc	aattcagt	ttt	cacttagaca	aaaatgccgc	cgaaattcga	60
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tgcaacttgc	ccaaaagg	ttt	ggccacttgg	atttgtc	ccccat	aaaaaaattt	180
tgcaagg	acacaggact	ggaaagg	ct	taagg	ttacc	tgcaagctga	240
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cgaacctccg	cgagaccgc	aaa	aaaatc	aaacgt	gaag	cacaatggca	360
cgagcaagt	atcaacattt	cgcgt	cagat	gcgc	ccctcg	tcaatcgac	420
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acatccgcac	gacattgtgg	acgc	gatc	agg	ggag	atcgaaatac	540
aagaaggac	gggc	ccctccg	at	ttt	gtgg	ccgaggata	600
ttgc	caattt	cattcattc	tcaattt	ttt	gtgt	ttgtgttgc	660

&lt;210&gt; 50

&lt;211&gt; 625

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 50

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gtggcaatcc	gggacggcg	gtt	cccttac	ccact	gcctc	ctacaa	accg	atcccccgaa	120
tacatgaaca	tcgtgaccc	ccctt	tttcc	gtg	cccaatt	tcc	gcata	ctcgggcg	180
atcgagg	acagac	cccttc	gttgc	ccgt	taact	taca	acact	tttacca	240
ccctaccgca	actaccgc	ctac	acc	tttgc	gca	aatgt	ctt	gttgcacca	300
tacttctcgc	cgctgtacaa	acga	aggatc	tttccc	acc	tttgc	actgt	ttgtactat	360
aaagcgaacc	cgcactattt	gcact	acc	cac	ctt	tttgc	ggactat	ctaccagg	420
aatgttgc	actacgacaa	cccttcc	caat	tac	ccctt	tttgc	actaca	acc	480
ggatatgtc	ggccgtatca	ctacc	gtc	cat	gcgt	tttgc	cccacc	tttgc	540
gaaggaatgg	tca	ggaa	ac	gttgc	acaa	atcg	actgc	tccaaattt	600
attcgaaaaga	agacgaa	aa	ag	tttgc	tttgc	tttgc	tttgc	tttgc	625

&lt;210&gt; 51

&lt;211&gt; 402

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 51

gaattccaa	ttt	gagcaac	at	ttt	gaaaa	ttt	cccgagg	tttgc	60
cggaaactt	t	ctcaagcg	ca	gg	ttt	tttgc	ccgc	aaagcaaa	120
acaaattt	ttt	ttt	ttt	ttt	ttt	tttgc	tttgc	tttgc	180
agcagtattt	gg	gg	gg	gg	gg	tttgc	tttgc	tttgc	240
cgaagaaat	gg	gg	gg	gg	gg	tttgc	tttgc	tttgc	300
tcaaaggcat	ca	ata	aaat	tttgc	tttgc	tttgc	tttgc	tttgc	360
gtcagatcaa	ca	ac	ccgg	tttgc	tttgc	tttgc	tttgc	tttgc	402

&lt;210&gt; 52

&lt;211&gt; 433

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 52

ccgacccgta	catcg	tttgg	gg	ttt	atcc	gtc	tttgc	tttgc	60
aacgcggta	cgcc	aaagag	aagg	gac	gtt	ccat	gg	tttgc	120
agcgcgtt	gg	gg	gg	gg	gg	tttgc	tttgc	tttgc	180
ccgtcggac	cg	cactt	aa	ac	tttgc	tttgc	tttgc	tttgc	240
ccgtggggcg	gg	ttca	aa	gt	tttgc	tttgc	tttgc	tttgc	300
ccgcgaggac	caaa	atca	aa	tttgc	tttgc	tttgc	tttgc	tttgc	360
aagaaggaa	at	tgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	420
aaaaaaa	aaa								433

&lt;210&gt; 53

&lt;211&gt; 768

&lt;212&gt; DNA

AKK110P1

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 53

gaattcgttt	gaggtcaaac	tttattagc	tat	ttaacaa	tgtccgaagg	aggagc	aaa	60
aagagt	gcgggtccaa	gggggggtt	t	atgtcaaga	aatttgcgat	cgat	ttgcg	120
tccgg	gtt	ctgcggcggc	tgt	tccaa	actgttgg	ctccc	atgt	180
ctt	ttgc	agtgcaaga	tgcttccgc	cacat	cactg	ccgaca	aaacg	240
attat	tgac	tgcttgtcc	tgt	ccgaaa	gagcagg	ttctgt	act	300
aacttgg	cca	acgttatccg	ttat	ttcc	actcaag	tgc	tcaagac	360
ac	at	gcatctttac	ggagg	gac	ggactt	gtcg	tttcttgc	420
gtc	at	gtt	ggactt	ggc	tttgcacgt	cgct	atccatccg	480
ctgg	gat	ggccgtacgc	gtt	ggcc	tcgttgc	aaa	agctgt	540
tca	acgg	ggcccaactgc	atcg	ggcc	tcttcaagtc	ggacgg	ttccgcg	600
acc	gg	cttcgtctcc	gtccagg	tca	tcatcattt	ccgc	ccgc	660
gct	tgac	ac	cgcaagatg	tcc	ccgatgg	gcagat	aat	720
cat	ggcc	at	tttgcgc	tc	ggatgg	gaat	tttccctca	768
cat	ggcc	cg	cgtcagg	tgt	cgtccgg	cct	ctcc	

&lt;210&gt; 54

&lt;211&gt; 338

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 54

gaattcc	agattaattt	gaatgg	tga	gaacat	cgaa	gagatt	tttgc	ccgaaat	60
cgg	tttcc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	120
ttt	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	180
aa	aa	aa	aa	aa	aa	aa	aa	aa	240
cg	cg	cg	cg	cg	cg	cg	cg	cg	300
cg	cg	cg	cg	cg	cg	cg	cg	cg	338
aa	agg	agg	agg	agg	agg	agg	agg	agg	

&lt;210&gt; 55

&lt;211&gt; 267

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 55

gaaatt	tttgc	ccgat	tccag	cgaca	aggat	tttgg	agg	cggtc	gac	aatt	tgac	gag	60	
gac	ggc	120												
tg	gaa	aa	at	tc	aa	tttgc	ggg	at	ggc	ggg	ggc	ggc	ggc	180
cc	at	at	tttgc	240										
cg	tttgc	300												
tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	360
tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	420
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tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	600
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tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	720
tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	tttgc	768

&lt;210&gt; 56

&lt;211&gt; 597

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 56

gaatt	cg	gac	act	tcg	at	ccgg	at	tttgc	60						
cat	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	120
ac	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	180
ac	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	240
cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	300
cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	360
cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	420
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cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	600
cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	660
cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	720
cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	768

&lt;210&gt; 57

&lt;211&gt; 80

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

## AKK110P1

<400> 57  
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 ttccgtacgg gccccgtcgtg 80

<210> 58  
 <211> 513  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 58  
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 gncggctcgg caagaaagt gaggaca**CC** cgaagtcgtc gaagactggc gacgcccggaa 120  
 ttgtcgaact gattccgacc aagccgtat**GT** gtgtggaggc attcactgtac tacgcaccgc 180  
 tcggccgttt tgctgtcgc gacatgagg**C** anactgtgc cgtggccgcg atcaaatcag 240  
 tggagaagac ggaaggccgtt gccaaggat**GA** ccaagccagc gcagaaggtc ggcgcgactg 300  
 gtggccggaa gaaagacatga ccaaggggag gggcggttcc ctaaggggca accgtcgacg 360  
 aaaatgcac caaccttctt tttatcgat ttttattcag ttcccttccac ccgtctctat 420  
 ccatattgtc gtgtcggttgg ataatgtttt attttttgtt attgtccctgg ttggaaaata 480  
 aatttggtca attaaaaaaaaa aactcg**GC** gaa 513

<210> 59  
 <211> 393  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 59  
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 aaaaggggca agggcttgat caaggtcaat gggcgccctt tggactacat gcagccggag 180  
 attctgcgc ttaagctcca ggagccattt ctatgttgc ggaaggacaa atttgaggga 240  
 atcgacatatac gaatccgcgt caagggccgtt ggacacattt cgcaatttt tgcaattcgc 300  
 caagcattgg ccaaggcaact ggtcgcttcc taccagaaga atgtcgacga gcagagcaaa 360  
 aaggaactga aggacattt tggtgttac gac 393

<210> 60  
 <211> 154  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 60  
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 taagaaataa tttttagat caaatgtttt gatgtatgc ctgtttttt ttgtgtataa 120  
 aaaaaatataa taaaaaaaaa ccggccgata**C** tgac 154

<210> 61  
 <211> 666  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 61  
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 aactgtcatc atgcaaaattt tcgtcaag**AC** gtcaccggc aagaccatca ctctcgaggt 120  
 cgaggctagc gataccatcg agaacgtgaa agccaagatc caggacaagg aggccattcc 180  
 gcctgatcag cagcgctga tcttcgcggg aaaacagttt gaagacggac gcacccggc 240  
 cgactacaac atccagaagg agtccactt ccattctcgat ctgcgtctcc gtggcggaaat 300  
 gcaaatttc gtcagacgc tcaccggcaaa gaccatcaact ttggaggatcg aggccagcga 360  
 caccatcgag aacgtgaaagg ccaagat**CC** ggacaaggag ggcattccgc ctgtatcagca 420  
 gcgctgtatc ttccgcggaa aacagctcgat agacggccgc actctggccg actacaacat 480  
 ccagaaggag tccactctcc atctcgat**CT** gcgtcttcgtt ggaggagaga actgaatcgc 540  
 gggctgtatgg aaagatgacg aatatgtat**GT** ctattcgatg acttgtctt ttgtatataa 600  
 ttgtattgtt tccatttgc ggtcatcaaa tctttatgac cccctcattt ggcattggaaac 660  
 gataaa 666

## AKK110P1

&lt;210&gt; 62

&lt;211&gt; 213

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 62

gaattcgttt gagaaaacttt ttcaaccatt cattcaaatg tctcatcaag tgacacgggc 60  
 agcaactcaac cacgggacgc gtgtactgag cgtgttggag aaggtcaagt tggctcgctg 120  
 gttttagggag acacattcg tgcgcagaat ggctcgaaga taccggcag aatttggat 180  
 ggaaccaccg cagttggacc aagtgaagaa gtt 213

&lt;210&gt; 63

&lt;211&gt; 488

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 63

agcacccggct caatcccaa tggcacaacg acggcattct cgggcattagg agacggagtc 60  
 ggtctggag aacaacagcc aattcccgtc gtaagcgatg cgggactgga tgccgaagaa 120  
 cagctgagaa tggccagaat gtgagccgga ggacctgaag atttatgaac gaaatttcc 180  
 agtgaagtgg accaacgctc ttgcactttt a tctgctttgt gtaaagtgtt tagaatcgcc 240  
 ttccaattca aaggcttttcc attcccaaaac ttttattttt gcgaaaaaaa ttcttagga 300  
 taagcgtgaa taatttatttgc atttgcattt tctttctttt atctccgcct cgaagtcgca 360  
 agtgtccctt ttggcccggtt ccctttgtt ttgaatgtt ttccattttcc atccccctcac 420  
 ttctctat ttgtgacatt cagctgcatt gttcgactcc cattttaaaag ttgagtgaaa 480  
 tgcgattt 488

&lt;210&gt; 64

&lt;211&gt; 249

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 64

wccrgakbng aacahcdkgd vhwatnvcn gschvbwagc rnrgtcsvddb wgnhnsswtg 60  
 gkgdylrbwnt msnwrmancng artsstsgaa ttcccaagtt tgagagtaaa tattattagc 120  
 taaaaatggc agtcggaaag aataagagaa tggcaaaaaa gggagccaa aagaaggctg 180  
 tcgatccgtt cacacgcaaa gaatggtacg acatcaaagc gcccgcgtg ttcacacatc 240  
 gaaatssts 249

&lt;210&gt; 65

&lt;211&gt; 362

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 65

wcbrhdyb ytsgrsnck tbdsbhcsy gcdwkmtnvk hscngdckty nykkvbmr 60  
 ntmsnwrmancng rgartsstsg tcaaccgtac tcaggaaacg cgcaatttcga gcgactttct 120  
 aaaaggccgc gtttacgaaatgtcactggg tgacccaaac agcaactgacg ccgactttcg 180  
 aaagttccgc ctgatctgtg aagaggtaaa gggcaagtt tgccctgacca actttcacgg 240  
 aatgtcgttc actcgggaca aactgtgtt tattgtcaag aagtggcaca cgctcattga 300  
 ggcgaatgtg gcagtgaaga ctaccgacgg tttcatgctc cgactctttt gtatcggtss 360  
 ts 362

&lt;210&gt; 66

&lt;211&gt; 128

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 66

aatcaaatta agaagacgag ctatgcaaaaa gcctctcagg tgcggatgtat tcgtgccaaa 60  
 atggtgagaa tcatgcagaa agaggtcttcc tccggcgtat ttgaangaaa gtagtcaaca 120  
 agccgtat 128

## AKK110P1

&lt;210&gt; 67

&lt;211&gt; 502

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 67

gaattccatt	aaaaaactaa	acgaacaaat	ctaaagatgg	ccaccgaagt	ggagggaaaat	60
ttccctacgg	ttgaccatg	gggtgcgtg	gaggaagtgg	gtggtaaga	gtcgatgcag	120
ttggtagcc	ttgacgttac	cgaggtaaa	ctgttcggaa	aatggtcct	taacgatgtg	180
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gtggAACGGT	tgtctttgtc	aatgatgatg	cacgggcggg	acaacggaaa	gaaactaatg	360
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ccaagtgtt	gtcaatgtc	tgataaacag	tggggccnc	gaagattnca	cacgtatcgg	480
acgtcgggc	actgttcgtc	ga				502

&lt;210&gt; 68

&lt;211&gt; 519

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 68

gcaaaacttt	atcaaataaa	aaattttat	ttgccaaaca	aattttatgaa	aaaaaattca	60
ttaatcatta	aaactacatt	aaaaatatac	tttttagaga	atgtcgctt	aaatattctt	120
ttctccctt	tatgcattca	tctaaccaga	cttggaaagca	atatggctaa	tcaagtcaac	180
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agtcttaatt	gcattcttaa	tagccttcctt	cgttgctggc	ttctccaaac	gagcagtcaa	420
atcaacaacg	aaaacgtttt	ggcgtcgca	cacgaaaagc	cattttccgt	aagttccca	480
tccaattcat	ggattgacct	ttccaacagc	cttgcagc			519

&lt;210&gt; 69

&lt;211&gt; 218

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 69

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actgtttga	agcgaaggaa	agtttagggct	gctcagcgtg	tttctctact	caagaataaa	120
ttggagaata	ttaagaaggc	taaggtaaaa	acgcaagtt	tctttaaacg	tgctgagcaa	180
tacttgattt	catatcgacg	taagcaaaag	caagagtt			218

&lt;210&gt; 70

&lt;211&gt; 293

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 70

taagaaagca	gggaattttt	atgtcccaga	tgaacctaaa	tttgcttttg	ttgtgcgtat	60
taagggaaatc	aaaaagggtt	atttaaat	gctataaagt	tttagatggg	tttagacaat	120
tcttccttt	taatgccttc	taacttttc	aaaaaaagtta	tgattttatac	acccattaat	180
ctacaaattt	tttaattttat	catatccatc	ctcgccctcg	aaaagttctt	caacttttcc	240
gcttgcgtca	aatcaacaat	ggagtttca	ttaaattgaa	taaagctaca	atc	293

&lt;210&gt; 71

&lt;211&gt; 422

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 71

aatgcattta	agactgcttc	ggaaggaaaag	cttaaaggga	ttcttgata	tactgaggac	60
caggttgttt	ctaccgactt	tcttggcgac	actcattcgt	ctatttcga	cgccgaggcg	120
taagtttga	ttttctaaga	ttatatttaa	ccttttaat	ttttcagct	tatgggtctc	180

AKK110P1

aacccgcatt	ttgttaaattt	ggtagctgg	tatgataacg	agtttggta	ttcctgccgt	240
attgttact	tgattagcca	tattgcttcc	aagtctgggt	agatagatgc	ataaaggggga	300
aaaaagaata	ttttagacga	cattcttaaa	aaagtatattt	ttaaatgttag	tttaatgtat	360
taatgaattt	ttatccataaa	atttgggggg	caaatataaa	ttttttat	gataaaaagtt	420
tg						422

&lt;210&gt; 72

&lt;211&gt; 374

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 72

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tgagccagg	aaactggact	tcaacgttcc	gcttattatgt	gattgggctg	ctgtttctga	120
gtggcctcaa	gaagagggaa	ctcagggtgc	acctactgca	ccaattggtc	agccacagcc	180
tcaacagcag	caaactcaac	aaggaggtga	ttggaactct	ggtactatgt	gatggtaag	240
ggcagaaaaa	ttgatagaaa	gagaaattat	tatggaataaa	atgtaatcaa	tgttgggtgc	300
tgatttattt	gttacatata	caacaagtcc	tatittgttg	tttatttaat	aaagttgttt	360
aattaaaaaa	aaaa					374

&lt;210&gt; 73

&lt;211&gt; 120

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 73

tttttttttt	tttttcttca	tcaatattttt	gaagtgaaga	accagaagta	gttgcatcg	60
agcttcaaa	ttttgttttt	tgattactt	ttaaacaaga	ttcaactgat	ggatctactg	120

&lt;210&gt; 74

&lt;211&gt; 369

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 74

gtctaaacaa	tcttagagcta	ttcggttcgt	ctgtctgttg	attatttagat	gttgattgaa	60
cagcactagt	ctctgtatgt	gttttcttca	atctcatttt	taagtgtatgt	agaggaagtt	120
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cagaccgcgt	cccttttaact	gtgtatctt	aagaaaaacc	tctaggcaac	gtcccagttc	300
cactcaaattt	caattttgtt	aaattttgc	cagatctaag	tccttcttcc	ttttgaacgaa	360
attgaactg						369

&lt;210&gt; 75

&lt;211&gt; 529

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 75

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aatctaaata	aggctctatt	ctaagttat	atttttctt	tacataaacc	gtcaaccctc	120
caagtttttc	aatgttgg	ggttttaat	gatccctctgg	taataatttg	taggctagaa	180
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tttccaaggc	taaatgcccc	aaattgaaa	ggactaaattt	aacgagtctt	aatgtttcat	480
taacaacagc	atttgtataa	attaatttt	gtctgtgttc	caaactaat		529

&lt;210&gt; 76

&lt;211&gt; 449

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

AKK110P1

&lt;400&gt; 76

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gaaaggcaca	acgacttaaa	caagaaaa	C	aggaagcgc	agctgaaatt	gacaaatata	180
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ttgctgtctaa	taaacagcga	gtaatttt	C	gtctacttc	acttgctgt	gacattcg	360
cagaactgca	tcacaattt	caacttca	C	ttaagctta	tgaaaagcct	gcctaattt	420
tagttgattt	attataaaaa	tgaaattga					449

&lt;210&gt; 77

&lt;211&gt; 643

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 77

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gaagcgttt	gccgctccaa	agaatttgg	t	gtggacaaa	ttgggtggag	ttttgc	120
acgtcccatt	tgcgccc	acaagctt	C	tgaatcg	ccttttattt	tgttcttcg	180
taatcgctca	aaatatgcac	aatcttataa	A	tgaagctagg	atgatttgc	aacaacgt	240
cattaaagtt	gatggcaagg	tgcgtacaga	A	aatgcgtt	ccagctggat	ttatggatgt	300
ggtttccatt	gagaaaaactg	gcaagtct	T	tcgttcttc	tatgatgtca	aaggacgtt	360
cattactat	cgcataaaaa	aggaagaagg	G	tcagcttaaa	ttgtgcaagg	tagtaaagca	420
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&lt;210&gt; 78

&lt;211&gt; 584

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 78

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aaataataa	ttgaatata	ataaaaat	G	aatttggagg	caaaaagagc	aattaatcg	180
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cattgaatgg	ccccaaacac	aaaaaaaat	A	attttggaa	gaaggggaaag	tagaagaacc	360
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aaaacatcaa	cataactt	cagatcaga	A	gcagttaaa	actgacaaac	aataaaaat	540
atgaattatt	taaaaattttt	tttaatgat	C	tttttaattaa	aatt		584

&lt;210&gt; 79

&lt;211&gt; 556

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 79

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gaaaggttca	cgttcattt	gtcgtgtcg	G	gaaagggtcg	tgctcaa	cctaagg	300
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gtcgcttac	caatgttg	acttctgg	G	cgggacgccc	tgcgtgg	ccct	420
ctgcataaga	gaatgg	atcttgat	A	atgtatgg	atataat	ttaata	480
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ttttagatca	gttact						556

&lt;210&gt; 80

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accatctcc

429

&lt;210&gt; 86

&lt;211&gt; 435

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 86

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 aattttcttt tcatacatttt ttaattttaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa agaacaacaa 120  
 acataattgt ctcctttttt ttataaaaatt taaagtttaa taagtttaa aacattctcg 180  
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 aaaaaaaaaa ttgcacaagt agcgagaaga tatcgagcag aaaaaaaaaa ggaaccccca 300  
 catatggatt tagttaaaaaa attacatcaa cgtttctca atactggttc tggttctaat 360  
 gggaaatactg aacattttga agttaatcca acaatggaaa catcgacatc ctcaacagag 420  
 ggtgttagcag atccg 435

&lt;210&gt; 87

&lt;211&gt; 501

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 87

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 atttggccctt ctaaagaggg ccgtttgggt ttgggtttttg tacttcagct gccttccacc 180  
 aattgttccct tagccaccaa atccgtaaaag agtacgtccct tggcggttca acgcataagac 240  
 gacgttccatg gctgtgaccg tctttctttt ggcgtgtacg caataagttt ccgcgtcgcg 300  
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 aatacgtttc actccaccac gacgtgccaa tcggccggatt gccgggtttttg tgataaccttg 420  
 gatgatatca cgcaagactt ttcggggcg cttagcgctt cccttccaa gtcctttcc 480  
 gccttttact cgtccggaca t 501

&lt;210&gt; 88

&lt;211&gt; 270

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 88

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 agaactatgtc tcgagttttt tttttttttt ttttaanaaa ttaacaattt atctcattttt 180  
 cctctccat gaaaattaaac aaaaagacga caacttaatc ccataattaa catcattttt 240  
 aagcttcagt cggcatgctt cgaataatgt 270

&lt;210&gt; 89

&lt;211&gt; 286

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 89

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 agtttagctt ttccagaacg aagagtcttc aacgtctgct tggccaaacca acaataacttg 180  
 cccgatttgg taaccatggc gagacgagca ttgatattttt ctgtggactt ttctgtttt 240  
 ccaacaacca ttgttaacgca aaattaaaaat ctctttttta acaaataat 286

&lt;210&gt; 90

&lt;211&gt; 391

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 90

## AKK110P1

&lt;211&gt; 424

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 80

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ctac

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&lt;210&gt; 81

&lt;211&gt; 89

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 81

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&lt;210&gt; 82

&lt;211&gt; 168

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 82

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ctcgctccaa ttcgtccctc ttcttgataa gatataattt gctcgaac

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&lt;210&gt; 83

&lt;211&gt; 67

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 83

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ccagtagc

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&lt;210&gt; 84

&lt;211&gt; 42

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 84

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taacacgacg aagaggcgaa acatcaacaa cctgacgacg aa

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42

&lt;210&gt; 85

&lt;211&gt; 429

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 85

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gatatcgctt aaagaccatt taccacaaa ttatattca ggaaaatcaa ttgtatgtcat 360
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gacggatgct	tattggtagc	accgttattt	ttattttcg	cctatataca	aacggtcaat	360
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&lt;210&gt; 91

&lt;211&gt; 131

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 91

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attacacatc	a					131

&lt;210&gt; 92

&lt;211&gt; 571

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 92

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cctcaaaaaa	ttcattttatt	gacgacccagc	agcagggtgt	tgctgctgtt	gttggaccacc	180
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&lt;210&gt; 93

&lt;211&gt; 671

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 93

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&lt;210&gt; 94

&lt;211&gt; 289

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 94

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289

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 <212> DNA  
 <213> Meloidogyne incognita

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 aatggccgaa gtgcttaag gagtggact tgaactttac gattgtgcct tggcaaattct 240  
 tatagctgtc gagccagtc cg 262

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 <212> DNA  
 <213> Meloidogyne incognita

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 tcaagatagt tataatgtgg gga 323

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 <213> Meloidogyne incognita

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 gaagtggata gaaataagaa caagactttt caatgtcttt tccaaatagaa tcaggaatta 360  
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 <212> DNA  
 <213> Meloidogyne incognita

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 aaaaagaagg atggcttcga tgccaaaaag ttgtcgattt atttggcttc tggaggaact 180  
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 <212> DNA  
 <213> *Meloidogyne incognita*

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<210> 100  
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 <212> DNA  
 <213> *Meloidogyne incognita*

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 <212> DNA  
 <213> *Meloidogyne incognita*

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 tttggactgg tttagtaacc aattgaagaa tcaaggttaa atgagtctaa ataaaaatgg 180  
 agagggggaaa gaggagaggt taattttttta agaaaaaaa 219

<210> 102  
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 <212> DNA  
 <213> *Meloidogyne incognita*

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<210> 103  
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 <213> *Meloidogyne incognita*

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 actggaaagg cttaaagggtt acttgcaat tgactatcca aaaccgaatt gcca 114

<210> 104  
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 <213> *Meloidogyne incognita*

AKK110P1

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gacccgtttc	aatggcggaaa	aaaattggaa	gggactgtta	aggaaaattct	tggcaactgca	180
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<210> 105

**<211> 571**

**<212> DNA**

**<213> *Meloidogyne incognita***

<400> 105

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<210> 106

<211> 235

**<212> DNA**

**<213> *Meloidogyne incognita***

<400> 106

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<210> 107

**<211> 702**

<212> DNA

**<213> *Meloidogyne incognita***

<400> 107

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tattttgcac	ttaaattttg	gaaagtgcac	aaaattgcct	ttctgagaat	tttttattttt	660
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<210> 108

**<211> 423**

**<212> DNA**

### **<213> *Meloidogyne incognita***

<400> 108

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agaaagaaa	tgccaaagga	gatgaagaac	ttgttgaaga	aaaagttca	aaaatatcaa	180
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AKK110P1  
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 aattatgagg ttgttgtgt tcctgacgtt tttgattgtc tggagctggg tgaggatcac 420  
 caa 423

&lt;210&gt; 109

&lt;211&gt; 994

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 109

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 gtc aaaaacta gccaaggcga aatttgcgc tttaaaacgt tcaagagaag agcaaaaaaga 180  
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 gaaattcaga taaaatataa aaaaaggatg ttataaataa agctgagg ttttgcgtt gcccgtatcg 480  
 accccaaaat ttgttgcgtt ttacagaaa ttgttgcgtt tttaaagaaa tatagaatgt 540  
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 aatttgcgtt cccagatgtat ttgtcgccag ctgcaatgtt acatgttacc aaaatatttgc 660  
 cttcaactgc taccctact caatgc caaa ggttttataa ttgttgcgtt ttgcacgtt 720  
 ttgcagatga tatttgcgtt tttaaaaattt acatttccat atgtatcaat gcttattttaa 780  
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&lt;210&gt; 110

&lt;211&gt; 476

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 110

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 ttatattcga aaaaatggct gagaatataa aagaaatcc tggcgaaattt gacggctctc 120  
 aaatttggatgat gatcaacgt ttcttcgata tggggatggat ggttatattttaa 180  
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 tcaaggattt actccacgaa atccggcccgat acctcagcgtt taaagactt gatgcc 476

&lt;210&gt; 111

&lt;211&gt; 189

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 111

cgaggacggaa agcgaaaaaa ttgaatttgcgtt agaattttggt gatataatgg ctggagagac 60  
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 ttggaaatgtt cccaaaatttgcgtt ctttttgcgtt aatttttgcgtt tttaacgtt aaataatgtt 180  
 taaatgtt 189

&lt;210&gt; 112

&lt;211&gt; 164

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 112

ttggggaaat tttaattttttt aaacaaatattt aataattacc aaacaacaaa aaagaatccc 60  
 aaaaacaaca tttttaatc aatgtacaga catatatttgcgtt caataacgtt gtgtggattt 120  
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<210> 113
<211> 539
<212> DNA
<213> Meloidogyne incognita

<400> 113
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agtcagtaaa agcctcaaca cacattggct tggttggaaat taagtgcaca ataccagcat 180
ctccagttctt caaagccttt ggattgtctt caaccttctt tccagttcga cggtcgaccc 240
tctctttaag ctcagcgaac ttgcaagcaa tgtgagcagt gtgacagtcgca agaacaggcg 300
tgtagccagc agcaatctgc ccaggatggt tcatgatgat aacctgagca gtgaattgcgt 360
tggctccctt tgctgggtca ttcatagagt cagaagtgac tgaaccacgt cggatgtcct 420
tgacagagat gttcttaacg ttaaaatccaa cattgtcttc aggaaacagct tcaggagag 480
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<210> 114
<211> 314
<212> DNA
<213> Meloidogyne incognita

<400> 114
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tccgtcgatt tactgagatt ggttctticta aatttgccca tcccgctttt gttccaagcc 120
cggagaatct tgaaagagta aggaaatgtc cagtttttgt ttttgggtgt ggtgngcttg 180
gatgttggaaat ttggaaaaat ttggccttat caggatttca aaatattgaa gttatgtata 240
tggacacaat tgacctttca aatctcaaca gacagttttt gtttcgtgaa cacgatgttg 300
gcttatacaaa agca 314

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<210> 115  
<211> 200  
<212> DNA  
<213> *Meloidogyne incognita*

<210> 116  
<211> 471  
<212> DNA  
<213> *Meloidogyne incognita*

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gttgcttatt	atcgtaattt	gcttggcc	gatccggagac	gtcaccggcc	aaagaagttt	360	
ggaggaccgt	gtgtcggtgc	tcgttatcag	aaatcttatac	gttaagaagt	atgaaattat	420	
aaaattgtgt	gttacgaatt	aattgttatt	ttgttggat	aatntgaat	a	471	

<210> 117  
<211> 593  
<212> DNA  
<213> *Meloidogyne incognita*

AKK110P1									
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aaaagatgtt	ctgggcagggt	tctggtgctg	attttgttat	tgatgcgact	ggagttttta				360
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<210> 118  
<211> 576  
<212> DNA  
<213> *Meloidogyne incognita*

<210> 119  
<211> 559  
<212> DNA  
<213> *Meloidogyne incognita*

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	tcaaaacttca	ttatccggct	attacagttaa	ccatctggca	cggaaaaactc	tcctgtactg	180
	gcaatagcaa	cgtctgttagg	tttgcaaaaaa	tgttttgc	ctgtcccttg	aacaagttt	240
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	acgtcagtaa	cccaactatt	gccgtgggca	tcgattgtt	gtccatgagg	catgtaaaac	360
	atgctttttc	cgtattcttc	caagactgtcc	cctgattccg	tgtctataac	agcaattgtt	420
	gttggggaaa	tgatggccag	ggatctgttt	aggtgttgt	tctcatcaaa	cggaaaatttca	480
	tcccaaaactc	tgtcagatcg	gtgaaaaaaga	acaagtgcgt	tcaatggatc	caatgcataa	540
	cccgaaatctt	cccaatat					559

<210> 120  
<211> 366  
<212> DNA  
<213> *Meloidoqyne incognita*

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tgctaaacaaa	attgtgccaa	attcaatcac	aaaggccacag	gcaaaaaccga	acagcacattt	300	
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ggagga						366	

<210> 121  
<211> 661  
<212> DNA  
<213> *Meloidogyne incognita*

<400> 121

## AKK110P1

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aagggttacg	taaatcgaat	accgactgtg	gtatcttaat	tttccatga	aattctccaa	540
aaaaaaaaaa	ttttttttat	ttttttccca	taatgtcatac	tatatttttt	gcttttaatc	600
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a						661

&lt;210&gt; 122

&lt;211&gt; 173

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 122

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ttccttgc	cacactctct	aaccctcatt	gaagatctca	acttacttt	tgt	173

&lt;210&gt; 123

&lt;211&gt; 584

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 123

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&lt;210&gt; 124

&lt;211&gt; 650

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 124

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&lt;210&gt; 125

&lt;211&gt; 1013

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 125

## AKK110P1

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&lt;210&gt; 126

&lt;211&gt; 80

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 126

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&lt;210&gt; 127

&lt;211&gt; 585

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 127

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&lt;210&gt; 128

&lt;211&gt; 287

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 128

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ttcgttatga ctctcgAAACC ggtcatcaca acatgtacGG tgaatac 287

&lt;210&gt; 129

&lt;211&gt; 175

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 129

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## AKK110P1

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&lt;210&gt; 130

&lt;211&gt; 599

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 130

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&lt;210&gt; 131

&lt;211&gt; 466

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 131

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aattgcacca	gccatccggc	gagatccatgt	acttatttgc	atagcatttgc	gcattcaccat	300
acgctacatt	gaggcgttaat	tgacttcaga	atcccgaaatttgc	gttctgttgc	tgcagatatac	360
tccctgttaggt	ggtagggatgc	ggctgtgc	gcccataatatac	atgggatca	gcatttgc	420
gataggtgtat	gcccagatag	ttcaagatgc	tctgtataacgc	ctgggg		466

&lt;210&gt; 132

&lt;211&gt; 266

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 132

atgaaatccg	agtttttgc	atcaaggccc	gtgaaaattttgc	tctttcgca	ccttattttgc	60
tggaaatgg	agcgcggcgttgc	aagattttgt	gcgatattca	cggtcaatac	aacgaccc	120
tgcggcttttgc	tgaatatgg	ggttttccgc	ctgaagcgaa	tttattttttgc	ttgggtgatt	180
atgtggatag	aggaaagcag	agtttggaga	cgatttgc	gctgttgc	tacaagatca	240
aatcccccg	aaatttctttgc	tgctga				266

&lt;210&gt; 133

&lt;211&gt; 308

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 133

tctatcaacc	aatatataatgg	attttacgt	aatgcacaaac	gcagattttc	tataaaatgc	60
tggaaaacat	ttactgttttgc	cttcaatttgc	ctgccaatttgc	ctgtgtgtat	cgatgagaaa	120
atattttgttgc	gccatggagg	tttgcacca	gattttgcaga	atatggagca	aatttcgaaga	180
attatgcgac	cgacggatgt	gccagatatac	ggctttcttgc	gcgcaccc	atgttctgtat	240
ccagaccaag	atgtccaaagg	attggggat	aatgatcg	gggtcttgc	cacttttgc	300
ccagatgt						

&lt;210&gt; 134

&lt;211&gt; 335

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

## AKK110P1

&lt;400&gt; 134

taaatttagt	ttctttctt	ccatctttt	ttatgtttt	aaagagtgt	ccaaaacaaa	60
tggccccc	tgatggaga	agcaggaaa	attattaca	agaacatca	attcctcaac	120
tttttgggg	tttaatgact	ggacitata	acaatcaacc	aatcgatct	attcaattt	180
tggagaatgc	aatagctaaa	tttcgaaaaa	atccgtatct	tccattaaag	tgggatactt	240
ttataaagtgt	ttcgccctaa	caacagcaac	aacaacagac	gagaatgaat	actggagaaaa	300
atgcagttt	ttataaaca	agactccta	tcgaa			335

&lt;210&gt; 135

&lt;211&gt; 506

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 135

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atccacaaac	ataatattat	tgaactttt	ctttttaaa	cttatcaag	gccttcttt	120
ttcctgagac	tttgatcacc	ttcaaaaat	taaaacgaac	agttttactc	aaaggcctgc	180
attcaccgat	cgtgacaata	ttccaatag	agatatcacg	gaaacatggc	gaacagtgaa	240
cggacatgtt	tttgcgtatc	ttctcgat	gacgatattt	cggaacaaag	tgcaaataat	300
cacgcccata	gacaattgt	cgctgcattt	tgttcttgat	aacaacacca	gtcaaataat	360
ggccacgaat	tggaaacattt	ccagtggaa	gacactttt	gtcaatataa	ttgccttcga	420
tagcctcg	tggagtttta	aatcctaacc	caacatttt	ccaataacga	tccttatttt	480
tcggctntt	gccaatccct	tcgc				506

&lt;210&gt; 136

&lt;211&gt; 230

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 136

aattccctaa	actctgccc	ggctgtcc	ctcaaaacga	caccctcg	ttattatcac	60
ctccagtc	ctacgaaaat	tcttgcgag	atcaagggag	taattcgaca	ttatggattc	120
ttttgttgt	tttaattgt	ttattttgc	tactaattt	ccttctaatt	gccgcctacc	180
tccgttgtc	catttttggc	tccgccccct	acaaaaacca	gttccgtcg		230

&lt;210&gt; 137

&lt;211&gt; 216

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 137

acaaatcac	aacaacaaaa	tcattgtt	cccaaataat	ccaaaagttc	tcctccaact	60
tcactcg	tttgatccaa	ctctactagc	tgttattcg	ccttagctgt	gccgttaatt	120
tctagtgt	cgaagaaaag	tgatgaacaa	caaagacgg	gggaatggac	aaatctaaca	180
ttattaattt	tttattctca	tgattgtaaa	ttgcat			216

&lt;210&gt; 138

&lt;211&gt; 395

&lt;212&gt; DNA

<213> *Meloidogyne incognita*

&lt;400&gt; 138

atgcattcct	gaagcaattt	tgggtatgga	cattgtatgc	caagcaaagt	ctggtatggg	60
gaagacagct	gtatttgtt	tggcaacact	ccaaacattt	actccagttt	acgggacgg	120
ctctgtctc	gttatgtgt	acactcgcga	acttgcttt	caaatttcaa	aggaatatga	180
aagatttagc	aaatatatgc	ccggaactaa	ggtttcggtt	tttttgggt	gtatgccat	240
caagaaggac	gaggagactt	tggctaagaa	cactccgcac	attgttgtt	gcactccagg	300
gcgtctgc	gcgttgggac	gtacaggaca	attgaagctg	aaaaacatca	aattcttcgt	360
tttagacgaa	tgtgacaaaaa	tgattgggaa	cgctg			395

&lt;210&gt; 139

&lt;211&gt; 591

## AKK110P1

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 139

gaattcggcg ttgtctcggt gtccacgctc aatttcaccg aaatttttgg ggcaggcgtc 60  
ctccacacca aactctgggt cattgacaac cggcactttt atctgggttc agcaaacatg 120  
gactggcagt cacttactga agtcaaggaa atgggtctta tgctgttcaa ctgctcctgt 180  
ttggcgtggg aactgagcaa aatatttgcg atttactggc ggattggaca gaatcacaat 240  
cgcttgcggc ctgtttggcc agtttattta caatcaaaat tcaacgctca acacccaatg 300  
gaaattcatt ttggacctga gcccctcgac acgtacattt cgcaactcgcc tgagaagttg 360  
aacccaaagg gcagagaaca cgacccttcg gccatatgct catgcatggg aaaagccaaac 420  
gaatttggtc gaatttgcgtt aatggattat attcctgcaa caatttacat gccgaatggt 480  
aacaacatat attggccatc gatcgatgac gcgataagaa cggcagctta tcgggggtgtg 540  
aaagttgacc ttgggtgagt ctgtggccccc atttgaatga acgagcgatt t 591

AKK110P1

<213> *Globodera rostochiensis*

&lt;400&gt; 11

acccaggcac tctgttcatc ttccggatcg ctttttggca atgtcaacaa cactttgctg 60  
 gccatgttgt ttctacagca cacgcacacc gtcgtctta cagcgttcac ctgcacaaaa 120  
 aatgtgcgtt aatggcggaa t 141

&lt;210&gt; 12

&lt;211&gt; 37

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 12

gcgttgggtg caagctgtac acaaggtcgc ccggttt

37

&lt;210&gt; 13

&lt;211&gt; 161

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 13

gcgcgttcca tcgccccac cacaaaaagt cccatcgctt catatcgtag cgcaaattgt 60  
 ctttggtgca aatggcggaa cggccaaaat aatggtcgg gccgtacaca accgcccacgg 120  
 ccacagcgcc aaccccacac caaatgcgg aattatcgaa a 161

&lt;210&gt; 14

&lt;211&gt; 306

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 14

gaattcggtt gagggtataaa taaaataataa atggcagccca acgaatcgct aaatgtggac 60  
 agtttgcata ctcgattgtt agaagttcgg gttgttagac cggaaaaaac agtgcggaaatg 120  
 gacgaatctg agatacgcac ttgtgcac c aaaaacacgtg aaattttgtt gtcgcagccca 180  
 atcttgcgg agctcgaggg acctttaaaaa atttgcgg acatttcacgg acaatataat 240  
 gatcttcga gattgttcga atatggggg tttccaccgg aagcgaacta tctatttctt 300  
 ggggac 306

&lt;210&gt; 15

&lt;211&gt; 261

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 15

gcaaaggctt gagacgattt gtttgcgtc tgcttacaag attaaatatac ctgaaaattt 60  
 ttttcttcgtt cgtggcaatc acgaatgtgc ttcaatcaat cggatttacg gattttatga 120  
 tgaatgcaaa cggagggttcc tcaatcaagt tggaaagac ttcaactgac tgcttcaact 180  
 gtctgcataat tgccgcttta atcgacgaaa agatcttttg ctgccccggg ggctgtctcc 240  
 tgatttgcata aacatggcag c 261

&lt;210&gt; 16

&lt;211&gt; 151

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 16

gaattcttg agtgcattca gcgtttaatt ttttcgtatt ataataagca tggctcgccg 60  
 accaaaaaag catttgaagc gacttgcagc accaaaaaaa tggatgttgg acaaattggg 120  
 tggcgttttgcg cattgtgcgg a 151

&lt;210&gt; 17

&lt;211&gt; 306

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